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INTERNATIONAL MONOGRAPHS

ASPECTS OF ANIMAL AND HUMAN NUTRITION

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This series of monographs on nutrition has been designed to present various aspects of the influence of nutrition on animal and human health and disease. It will concentrate more on aspects of health than those of disease.

Our chief concern is to study and show the nutritional environments best suited to a perfectly normal metabolism of the organism. From this object there will naturally stem descriptions of environments capable of creating a disordered metabolism: disease will be considered as a consequence and not a cause of this disturbed metabolism.

The title of an excellent book recently published is *Biochemical Disorders in Human Disease*. The theme of this series is: 'Human and animal disease as a consequence of biochemical disorders.'

These biochemical disorders leading to disease have numerous causes, of which the principal is nutritional.

The old saying 'Man is what he eats' might be rendered today in scientific parlance as 'Optimal functioning of the organism's metabolism is a correlation of nutrition'.

Another important object of the series is to bring out the influence of the soil and methods of cultivation on the biological quality of food products and, in consequence, on the metabolism of animals and men.

In modern dietetics and medicine it is often forgotten that our food products are basically manufactured by a single, unique machine—the soil—which plays an important part in determining whether the metabolism of plant, animal, and man will be well or poorly regulated.

The study of these many aspects of nutrition will pave the way for a synthesis of diverse disciplines: agronomy, plant and animal physiology, medicine and veterinary medicine, biochemistry, etc. These various branches, of interest in dietetics, have often been separately treated in watertight compartments. This has sometimes hindered the

progress of nutritional science, which is basically the resultant of all these individual sciences.

We thus hope to contribute to a better overall understanding of the ensemble of these problems by specialists in each of the relevant disciplines, and to show how nutrition is the principal factor determining normal metabolic processes or, in a word, health, in the animal and in man.

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Introduction

Trace elements are very small quantities of elements that are present in all matter. At one time they were regarded simply as contaminants, and were not studied with any great interest. Today this is no longer the case, and in metallurgy, chemistry, geology, and biology they are studied with great interest, for their significance is gradually being appreciated in many branches of science.

In this book an effort is made to restrict the field, and only trace elements known to be essential nutrients are discussed. It is unfortunate that the term 'trace element' is used so widely, as this concept covers such a multitude of elements functioning in such a variety of ways. Again, it is doubly unfortunate, as the term 'trace element' may give the impression that the element does not play an important role. In fact, the contrary is the case, and the trace-element nutrients play a most important part in many processes which they help to regulate, although they may only be present as one atom in ten million. The trace elements are anything but insignificant in their activities.

The literature on the nutrient trace elements is immense. It is scattered throughout journals and books written in many languages and upon many topics, for these substances are of importance not only in biology, agriculture, and medicine, but in subjects such as geology and soil science as well. As may be imagined, the scattered literature is not always readily available, and the specialized terminology of the various branches of science make it difficult to understand. In fact, the specialized terminology of modern science, which is almost incomprehensible to a non-specialist, is often a serious drawback to understanding present-day work, and it leads to compartmentalized thinking. It has certainly made it difficult to obtain a general picture of the significance of these elements in biology.

This book makes no attempt to be encyclopaedic. It is not a detailed monograph, and it does *not attempt* to discuss all the known biological implications of these elements. The aim of this book is quite distinct from this. It attempts to show the nature of the part played by trace elements in biology and to outline their importance in nutrition in all forms of life. Where possible, practical problems have been stressed, for it would be wrong to believe that these elements are only of academic importance. As will be seen, trace-element problems are of real economic importance and are widespread.

In discussing a subject like this one, where new research results are being published daily, it seems very important to stress *principles* rather than details which may become dated very rapidly. An effort was made to select experimental data that enabled a *coherent* whole to be depicted, so that trace-element nutrition appears as an integrated entity, not a mass of unrelated facts. But it must be admitted that here, as in all branches of science, a mass of contradictions, real and apparent, also exist, and only further research will show if this present interpretation is justified or not. But adequate references to the literature are supplied, and a brief list of books is included for any readers who wish to follow up any of the matters discussed.

As technical terminology can be understood only by a few, as little 'jargon' as possible has been used, although this does occasionally diminish the accuracy of statements. It is hoped that the resultant comprehensibility will compensate for this. Unfortunately, it is not always possible to eliminate technical terminology completely, but specialized terms are defined when they are used.

It is hoped that, in spite of its many shortcomings, this book with its biological approach to nutritional problems will stimulate and interest readers, and that this volume will enable them to appreciate the fascination that this branch of science has for so many minds.

I | The significance of trace elements in nutrition

Food and nutrients

The fact that food is necessary for life and that the nature of the food that an animal or human consumes is reflected in his behaviour and degree of well-being is well known. It is not always appreciated that plants and micro-organisms have the same requirements as man and animals. They also need food, and the nature of their food also influences their development.

But it is clear that the term food must cover a very large range of materials, because plants do not eat steak and onions, and man does not normally eat soil for the food it contains. Certain foods are reputed to be 'healthy', because they contain substances such as vitamins in small quantities, while others are regarded as undesirable, because they lack these nutritional factors. So in this book, although food will be discussed, a different terminology will be employed to prevent misconceptions. The term *nutrient* will be used.

All living organisms require a regular supply of nutrients. This is as true for microbes as it is for man. If there is a deficiency, or an excess, in fact if there is an imbalance of nutrients, then normal development will cease, and abnormal development will result. If the imbalance is severe death will eventually result.

Type of nutrients

Nutrients have a number of functions to carry out. One of the most obvious is to supply *energy* to the living organism. All forms of life need a constant supply of energy to stay alive, because life is always active. Thus, they need energy to respire, to reproduce, and to carry out the large number of energy-consuming activities which plants and animals undertake. The energy for all these reactions is obtained from *organic matter*, nutrients such as fats, carbohydrates, and proteins. These complex substances are broken down by the process of digestion, which usually reduces them to water-soluble substances such as sugars and amino acids. These latter can be used by the individual living cells as a source of energy.

The energy that is needed is obtained from these nutrients by the process of respiration, a complex chemical process whereby the sugars

and amino acids are oxidized in the cells and energy is slowly liberated. This oxidation is a 'burning' whereby energy from the food material is released and can be used to build up new protoplasm (i.e. living matter) or to carry out complex chemical reactions.

The amount of energy that living matter requires is large. Even plants, which are normally regarded as being inactive when compared to animals, utilize a great deal in growth. One has only got to see grass splitting a pavement, or a poplar tree cracking a wall, to realize how much energy is involved. And yet plants in general use up energy at a much slower rate than animals. However, they both have the same basic requirements. They need a continuous supply of nutrients, both organic and mineral, to grow and develop. All forms of life must replace the old tissue and build new ones, must reproduce, and must have a supply of energy for all biological activity.

Uniqueness of the green plant

The green plant is unique in one respect. It manufactures its own supplies of energy-giving nutrients, such as fats, carbohydrates, and proteins, from inorganic matter. It does this by means of the process of *photosynthesis*, a reaction in which light energy of the sun, carbon dioxide of the atmosphere, and water are converted into such substances as sugars, which are energy-rich nutrients. The plant builds these up into proteins, fats, and more complex carbohydrates.

Animals are unable to synthesize these food materials from simple substances such as carbon dioxide and water, and hence must have an outside source of these, as they do not possess a built-in supply as the plant does. And closer inspection shows that photosynthesis is undoubtedly the most important biological reaction on earth, because ultimately all life is dependent upon photosynthates. The old adage 'All flesh is grass' is perfectly true.

Because of this photosynthesis, plants are more *obviously* dependent upon the soil for mineral nutrients than are animals. The influence of mineral nutrient can be more readily studied in plants than in animals, and in this book many of the general principles concerning mineral nutrition will be illustrated with reference to plant experiments, because they are more readily conducted and are somewhat easier to interpret.

Essential elements

This *synthesized* organic matter, which contains carbon, hydrogen, and oxygen, forms the bulk of all plants and animals. In Fig. 1 an analysis of a maize plant is given, and carbon, oxygen, and hydrogen account for over 94 per cent of the dry weight [189]. If the atomic composition of a plant is studied, it is perhaps even more instructive (Table 1). The analysis of a human being shows a very similar picture,

the histogram in Fig. 2 shows that these three elements account for 93 per cent of the mineral composition of man [270]. *Carbon, hydrogen, and oxygen are known to be essential to all forms of life.* They are the essential elements that are present in the highest concentrations in living organisms, which is not surprising, since they are needed for

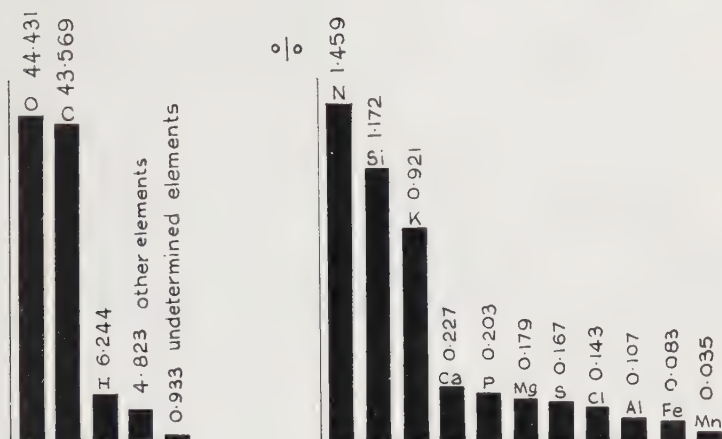


FIG. 1 AFTER MILLER [189]

The percentage of the various elements present in 'Pride of Saline' maize plants at maturity

Oxygen, carbon, and hydrogen comprise the bulk of the dry matter of the plant, and all the other elements combined constitute less than 6 per cent of the total. Essential elements like nitrogen are present only in concentrations of 1.5 per cent of the dry weight. Elements such as manganese and iron are present in much smaller quantities still, and hence are termed *trace elements*.

virtually all organic matter. Obviously, if life is to flourish they must be available in adequate quantities.

But other elements are equally characteristic of living matter, even if they are present in much smaller quantities. Nitrogen, without which there can be no proteins; phosphorus, potassium, and calcium are all elements that must be present and available if life is to occur. And they can all be readily identified in plants and animals without recourse to very refined analytical techniques.

But in addition to these readily identifiable elements there are a large number of elements that are present in only small quantities. Most of these can be found in the ash of plant or animal materials.

TABLE 1

The elemental composition of a typical plant (dry matter). From ZELLER [335]

<i>100 g. dry matter contain</i>	<i>1,000 million atoms of dry plant matter contain</i>
45 g. carbon	515,000,000 hydrogen atoms
41 g. oxygen	276,000,000 carbon atoms
7 g. hydrogen	188,000,000 oxygen atoms
2 g. nitrogen	10,000,000 nitrogen atoms
5 g. ash elements	3,760,000 potassium atoms
	1,840,000 calcium atoms
<i>5 g. ash elements contain</i>	1,740,000 magnesium atoms
2 g. potassium	1,060,000 phosphorus atoms
1 g. calcium	730,000 chlorine atoms
0.6 g. magnesium	580,000 sulphur atoms
0.45 g. phosphorus	340,000 silicon atoms
0.35 g. chlorine	320,000 sodium atoms
0.25 g. sulphur	130,000 iron atoms
0.14 g. silicon	3,000 boron atoms
0.10 g. sodium	1,000 manganese atoms
0.10 g. iron	300 zinc atoms
0.01 g. trace elements	100 copper atoms
	5 molybdenum atoms
	1 cobalt atom

This Table shows clearly in what low concentrations trace elements are present and effective. Thus, only 1 atom in 10 million in copper, one in 200 million in molybdenum and one in 1,000 million in cobalt. It is at such low concentrations that these elements effectively fulfil their nutritional role in biological systems.

Burning results in the loss of carbon, hydrogen, oxygen, and nitrogen, as well as a few other elements, but the concentration of remaining 'Ash Elements' is much higher than it was in the original matter, and so ashes can be more readily analysed for the remaining elements which are present in small quantities in the parent material.

In ashes the main elements present are usually calcium, magnesium, phosphorus, potassium, while a host of others are present as traces. Experimentally, it can be demonstrated that many of the elements named are *essential elements*, cf. Table 2, and in their absence no normal life is possible.

In the case of plants these mineral elements must be introduced from outside in the form of salts. They are definitely *nutrients*, even if they do not conform to our preconceived notion of foods. As all these nutrients enter the plant from the soil, and were at one time part of the soil, they are termed *mineral nutrients*, to distinguish them

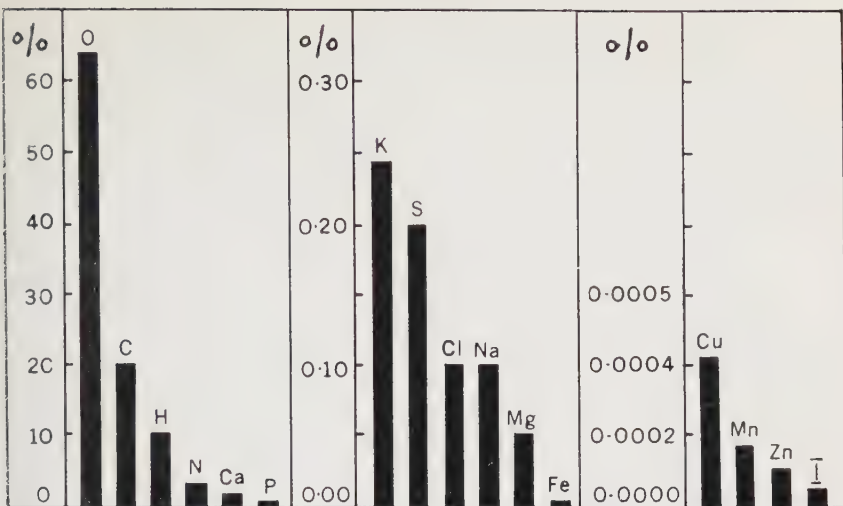


FIG. 2

AFTER SCHWEIGART [270]

Percentage composition of a human being

The concentration of elements in a 70 kg. [154 lb.] human being. Over 98 per cent of the weight is accounted for by oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorus. Other elements, even if present in such low concentrations as iodine, can and do play a most important part in normal life and well-being.

from organic nutrients. These essential mineral nutrients include nitrogen, potassium, phosphorus, calcium, magnesium, and sulphur.

The trace elements

As well as the above-mentioned elements, traces of very many other elements, such as iron, manganese, copper, and a host of others, are always present in living matter. At first the presence of such small quantities of elements was considered as a form of contamination, as no known function could be assigned to them. But today many of these trace elements are known to play a part in biological reactions, and some are definitely known to be *essential nutrients*. These *trace elements* are not all essential elements, as far as we know today; but those that are, being essential, play a very important role in biology, and no normal development can take place in their absence.

A look at Table 1 will make it clear in what small concentrations trace elements are active [335]. Copper, a very important element, is present only as 1 atom in 10 million. Obviously, it is very difficult to investigate the part played by such low concentrations of elements, and it is not at all surprising that we are uncertain about the nutritional significance of some trace elements.

TABLE 2

List of Essential Elements for higher plants, mammals and man

HIGHER PLANTS			MAMMALS AND MAN		
*C	Carbon	} MAJOR OR MACRO ELEMENTS	{	C	
*O	Oxygen		{	O	
*H	Hydrogen		{	H	
N	Nitrogen		{	N	
P	Phosphorus		{	P	
K	Potassium		{	K	
Ca	Calcium		{	Ca	
Mg	Magnesium	} MINOR OR MICRO ELEMENTS (TRACE ELEMENTS)	{	Mg	
S	Sulphur		{	S	
			{	Na	Sodium
Fe	Iron		{	Fe	Iron
B	Boron		{	Cl	Chlorine
Cl	Chlorine		{	Cu	Copper
Cu	Copper		{	Co	Cobalt
Mn	Manganese		{	I	Iodine
Mo	Molybdenum		{	Mn	Manganese
Zn	Zinc		{	Mo	Molybdenum
			{	Zn	Zinc

* These are present in all organic matter.

This list gives the elements generally accepted as essential for life and proper development. The division into major and minor elements is quite arbitrary, and concentration of these may vary from species to species.

Other elements may be essential for certain types of plants and animals. Selenium appears to be essential for humans in very small quantities, for instance, but it is not yet generally accepted as such. More refined research techniques are necessary to determine if other elements which are present in very low concentrations are truly essential, or merely beneficial.

Not all the elements present in plants or animals are essential for life. Silicon, which is present in macro quantities in maize, cf. Fig. 1, is not. Maize can be grown quite well in its absence, but it is of value to the plant, as it contributes to its mechanical strength. In Fig. 3 [86] a large number of trace elements are shown as present in plants. No well-established function can be assigned to such elements as rubidium or lithium, but future investigations may reveal some nutritional role. This figure also shows the range of concentration of mineral elements found in plants. Not all animals and plants have the same mineral composition, or as we shall see later, the same mineral requirements. Iodine, an essential element for animals, is not regarded

as essential in plants, although it is always present, and boron, which plants require, is not necessary for animals, as far as we know.

The fact that an element is present, or even that it exerts a beneficial influence upon development, does not qualify it for the status of an essential element. Many beneficial elements are known, but organisms can grow normally in their absence. Silicon and sodium are two such elements, as far as many plants are concerned, while vanadium may be one in mammals.

Essential minor elements

Certain essential elements, such as zinc, molybdenum, etc., exert their influence at very low concentrations. They are absolutely essential for proper development and growth, yet they are required in very small quantities, and if present in excess can be poisonous. These substances must obviously play a different role in the organism than elements like phosphorus, nitrogen, or calcium, which are needed in much larger quantities. The *macro-elements* or *major elements*, which are present in large quantities, are mainly constituents of proteins, cell walls, or mechanical structures. They also play a part in complex chemical reactions, but the bulk of the element is present in the form of protoplasm or of structural components.

The other essential elements which are present in minute quantities, the *trace elements*, or *micro-nutrients*, or *minor elements* (they have many names, but none of them is quite suitable for all purposes) cannot have important structural functions assigned to them. There is just too little of them present. Their role is primarily *catalytic*,* although not necessarily exclusively so. They may be built into structural units or food reserves, as zinc is into insulin [271], but nevertheless, their main function in biological systems is to be a component part of catalytic systems, which control the chemical processes of organisms. Their absence can prevent important reactions from taking place, and hence result in abnormal development and even in death.

While it is possible to generalize about the parts played by major and minor elements in biological systems, it must be appreciated that no absolute differences in function can be demonstrated invariably. Nitrogen is a component of all enzyme systems that have been isolated, and magnesium, another essential major element, is also a catalytic element to a very large extent. As a result, all efforts to

* A catalyst is a substance which in minute amounts promotes chemical changes without itself being used up in the reactions to any marked extent. A catalytic reaction is one influenced by a catalyst. The bulk of biological reactions are catalytic, and thus catalysts profoundly influence all microbes, plants, and animals. The most important catalysts in biological systems are organic ones termed *enzymes*, which will be discussed in more detail in Chapter II.

separate the two groups of elements are arbitrary, whether they are based on concentrations present or on their functions. Elements such as iron are often considered as semi-micro nutrients in human nutrition, being present in much larger quantities than 'true' trace elements such as molybdenum, which are present in quantities difficult to detect.

Lower organisms

In lower organisms such as microbes and invertebrates, where a very varied selection of biochemical systems can be found, *a large range of elements have been shown to be essential for certain kinds of organisms.* This is not the same for higher plants and animals, which show a smaller number of essential elements, cf. Table 2, and these are amazingly constant for nearly the entire range. While sea squirts are known to require vanadium, as it is a constituent of their blood, other forms of invertebrate life have not been shown to need this element. Until recently, it was not considered as essential for higher animals, but more recent work shows that it can play a part in mammalian blood formation, although it is not clear if it is an essential element or merely a beneficial one.

It must be clear that in spite of the remarkable similarity between metabolic phenomena in both plants and animals it is impossible to draw up a list of elements that are essential to all forms of life. There is too large a range of types of living matter to permit this.

Assessment of essentiality

It is clear that certain elements occurring in low concentrations are essential for the life of plants and animals. Unfortunately, the quantities of these trace elements which different organisms require for normal development varies so considerably that it is not possible to define trace elements in terms of the concentrations in which they occur. Thus, the Blue-green Algae require about 100 times the amount of molybdenum that Green Algae need.

It is more enlightening to discuss mineral elements in terms of their functions. In this book the term *trace element* will be used in a restricted sense to denote elements with a nutritional role whose main functions are catalytic, even if they sometimes occur in fairly large quantities, as is the case in certain *accumulator* plants, that is plants which accumulate large quantities of these elements.

The number of elements that can be identified in an organism is very large, as is clear from Fig. 3. How is it possible to determine which of these is essential for life and proper development? This is by no means an easy task. The mere presence of an element, even if widely distributed in plants and animals, does not prove that it is essential, as far as we know. Thus, elements such as lithium and rubidium, which are very widely distributed, and yet as far as is

known, they are not essential for life. (It must be stated that one group of investigators believe that virtually all elements are present in biological material, and that they are essential for life, but some of

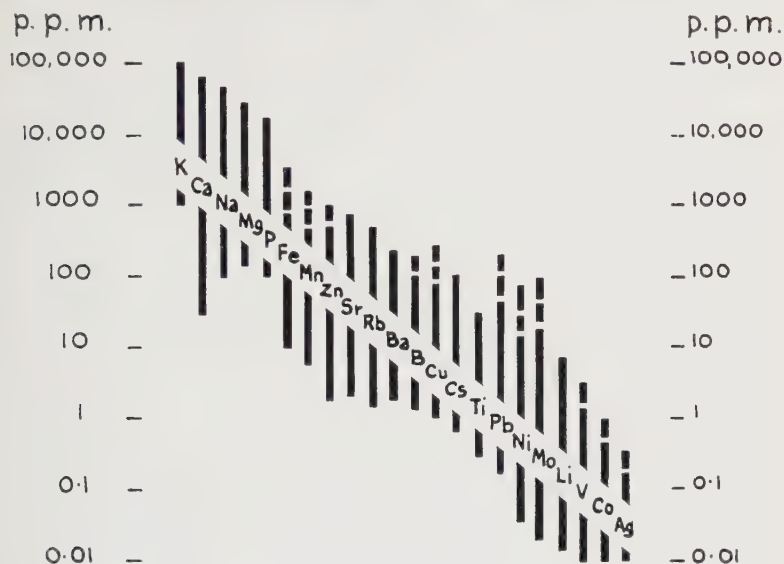


FIG. 3 FROM DE KOCK [86]

The range of concentrations of elements found in plant material

Not all plants contain the same concentration of elements, and there is a wide range even of elements such as molybdenum or cobalt, which are usually present in very low concentrations. There is a marked variability between the different species in their requirements for essential mineral nutrients.

them at unbelievably low concentrations, even for trace elements. This viewpoint must still be proved to be correct.)

In order to discover if an element is really essential, it is necessary to study the growth and development of plants or animals in the absence of the element being studied. As plants can be grown in water solutions made of pure chemicals, it is easier to study them than animals. It is very difficult to supply a really pure diet to animals, and unless vitamins and such other essentials are included in the correct proportions normal growth will not take place. As a consequence, more is known about the trace-element nutrition of plants.

If the absence of an element from a properly prepared water-culture solution results in either marked abnormality or death, then that element is deemed an essential one. The abnormality that results is a true *deficiency disease*, caused by lack of an essential element in its diet.

Experimental difficulties

Trace elements are frequently required in such low concentrations that it is not always easy to prepare solutions lacking them. Even the purest of chemicals are not absolutely pure and always contain some traces of these elements as impurities. Containers can contaminate chemicals, and handling may introduce other impurities. All these contaminants may be present in such low concentrations that our present chemical techniques may be unable to detect them. And yet, because the organism's requirements are so low, they may be present in sufficient quantities to fill its needs. It is thus almost certain that there are a number of essential elements whose existence is unknown, as the techniques to study them are not yet available. The type of practical problem facing the investigator is clearly seen in books such as Hewitt's [128], where he discusses techniques. The consequences of this state of affairs is clearly stated by Underwood [307] when he stresses what he terms 'the probably essential elements'.

Biological reserves

Another problem that confronts the investigator is that all living matter contains trace elements. In seeds they tend to be present in larger quantities than in other plant tissues. In humans and animals a similar state of affairs exists, and the foetus is usually rich in trace elements. This is particularly true of the liver, which in embryos and infants is very rich in such elements as copper.

It is quite impossible to deplete the stock of nutrients completely in living matter, and it may require growing the material for two or three generations on really deficient substrates before really acute deficiency symptoms set in. This is as true for animals as for plants, and it took Hill and his co-workers [134] three generations to deplete the manganese reserves of rats on a deficient diet so that the animals showed clear symptoms of manganese deficiency.

These reserves of minerals are of practical importance. In the field it may take several generations of deficient feeding to reduce the mineral reserves of stock to such a state that the *critical level* of nutrients is no longer reached. Then, and only then, do clear-cut deficiency symptoms appear. This problem, and the factors determining the critical level, will be dealt with subsequently when *induced deficiencies* of various kinds are discussed.

Because living matter is involved, it is impossible to obtain absolute deficiencies of any essential nutrient, as by definition, all essential elements must be present, even if at such a low level that no normal development is possible. This is an important point to grasp, because it means that *all deficiency conditions that occur are relative deficiencies*. As the organism's requirements may change with age, or with altera-

tion in environment, deficiency symptoms may appear where previously a healthy plant or animal existed. This will take place where the level of nutrition was previously considered quite adequate. Thus, sway-back (a copper deficiency) lambs are produced on low copper pastures,* which support apparently healthy sheep. But the level of available copper is not high enough to allow ewes to give rise to normal healthy lambs.

This fact, that all deficiencies are relative ones, is of great practical importance, as many widespread trace-element deficiencies are not due to absolute shortages of elements, but are *induced deficiencies*, brought about by altering the beneficial ratio of various nutrients to each other. This results in nutritional *imbalance*, with dire consequences, as will be seen later.

Problems with animal investigations

Because plants manufacture their own organic nutrients such as fats, sugars, amino acids, and vitamins, they are more readily studied than animals. Animals need a *continuous* source of organic nutrients, as well as of minerals. But it is extremely difficult to purify food materials to such an extent that clear-cut investigations into animals' trace-element requirements can be made. It is only fairly recently that good purified diets have been developed. And these are only of use for such experimental animals as rats. No purified synthetic diet that can be fed to herbivores like cattle and sheep exists. These are studied by feeding them fodder grown on deficient soils, and so deficiency conditions can be induced.

Much information exists about trace elements in animals, even the larger ones, but this information is derived from deductions made from more indirect methods of investigations. In most cases experimental procedure cannot be as well controlled as in plants, and so the information regarding trace-element requirements is not so clear-cut, and is in some cases contradictory. But the feeding habits of cattle, for instance, makes any other approach impossible.

Criteria of essentiality for elements

To set up useful criteria of essentiality is not easy. Those suggested as long ago as 1939 by Arnon and Stout [23] are still very useful. While originally intended for plants, they also apply to animals. Their criteria are still very useful, and briefly they are as follows:

An element cannot be considered essential, unless

(i) a deficiency of it makes it impossible for the plant or animal to complete the vegetative or reproductive stage of its life cycle;

* Other factors, such as high molybdenum, may also be very important, cf. Chapters VI and IX.

(ii) such deficiency is specific to the element in question, and can be corrected or prevented *only* by supplying this element;

(iii) the element is directly involved in the nutrition of the plant or animal, quite apart from its possible effects in correcting some microbial or chemical condition of the external medium.

An element fulfilling these requirements would be considered as essential in any branch of biology, be it medicine, microbiology, botany, or zoology. It must be stressed that a *beneficial* effect on growth or reproduction does *not* automatically qualify an element as essential, even if its effect is very pronounced. In this book the above criteria will be used.

II | Trace elements as catalysts

THEIR IMPORTANCE AS REGULATORS OF ENZYME REACTIONS

Catalysts and their characteristics

As long ago as 1836 the Swedish chemist Berzelius observed that minute quantities of certain substances promoted chemical reactions and increased the rate at which they occurred. He termed these substances *catalysts*, and the process whereby they stimulated chemical activity *catalysis*. Since their discovery, catalysts have been shown to be of great importance in all branches of chemistry: many important industrial processes, such as the hydrogenation or 'hardening' of oils for their use in margarine production, or the 'cracking' of crude petroleum are catalytic in nature. In biology, where most of the chemical activities are catalytic, it is virtually impossible to over-stress the importance of these substances.

Typical catalysts are needed only in very small quantities, and do not become parts of the product of the reaction which they promote. Usually they remain virtually unchanged after the reaction, so that very small amounts of catalyst can bring about very large changes. For example, one molecule of the enzyme *catalase*, the biological catalyst that decomposes hydrogen peroxide, *can promote the decomposition of 40,000 molecules of hydrogen peroxide per second at freezing point*. Like most other materials, catalysts can *wear out*, and must be replaced. This is particularly true of organic ones, as they wear out relatively rapidly, and thus they must be replaced continuously.

It must be stressed that catalysts do not initiate chemical reactions that cannot occur in their absence. What they do is *to speed up reactions* that take place only very slowly without them. These may be so slow that they are not readily detected. Hence catalysts bring about very appreciable changes. In many chemical reactions a supply of *energy* is necessary before the reaction will occur. This is the *activation energy* of the reaction, and this energy must be supplied before the reaction will start. Catalysts promote many reactions by decreasing the activation energy required, and so promoting the chemical reaction, which takes place more readily.

Enzymes—organic catalysts

In living matter a vast number of chemical reactions are taking place continuously, and virtually all of them are catalytic. As long ago as 1878 Kühne recognized that the catalysts present in living matter were different from those previously studied. He called these biological catalysts *enzymes*. Twenty years later Büchner, in a classical experiment, extracted some enzymes from yeast and showed that they were *non-living* organic compounds. This was important, as it led to the clear appreciation that these non-living enzymes were of paramount importance in regulating living processes. Büchner can be regarded as the real founder of that important but complex branch of science called Enzymology, which is the systematic study of enzymes.

As these enzymes are the catalysts that promote and regulate the multiplicity of chemical reactions in living matter, or to put it in another way, as enzymes govern and regulate the behaviour and functioning of living matter, it is absolutely imperative in all biological sciences to have some knowledge of enzymes. Since the trace elements are important primarily because they are either parts of enzymes or influence them, it is necessary to have a clear, although not necessarily a detailed or extensive, knowledge of enzymes to appreciate the biological significance of trace elements.

The composition of enzymes

Enzymes are organic catalysts manufactured by the protoplasm in living matter. The ordinary cell contains thousands of different enzymes, and these regulate the chemical activities which must take place if the cell is to continue to live. Being catalysts, they are present in very small quantities, and only a few have been isolated in a fairly pure state. It was only as recently as 1926 that Sumner first succeeded in crystallizing an enzyme, *urease*.

Today, although there is much we still do not know about enzymes, a great deal of information is available about these substances. Enzymes are proteins. That is to say they are very large molecules built up from various amino acids into long chains. These amino acids are organic compounds of low molecular weight possessing both amino ($-\text{NH}_2$) groups, which contain nitrogen and are alkaline in nature, and carboxyl ($-\text{COOH}$) groups, which are acidic. These chains of amino acids can be linked to other compounds, and thus a large number of different enzymes can be formed.

As far as we know, only a small number of enzymes are composed exclusively of protein and nothing else. The protein-digesting enzymes pepsin and trypsin are two examples. Yet even these can be influenced by mineral ions such as calcium or manganese in their

activities [68]. Most of the other enzymes that we know are composed of more than simple proteins. These are formed of *conjugated proteins*, complex proteins in which the proteins are linked to other non-proteinaceous substances. In such cases the entire enzyme system, or *holoenzyme*, consists of an *apoenzyme* (protein) linked to a non-protein *prosthetic group*. Sometimes the prosthetic group can be readily removed from the proteinaceous part of the enzyme, and then it is termed a *co-enzyme*. This type of enzyme is very well exemplified by *lactic acid dehydrogenase*, which converts lactic acid to pyruvic acid. The enzyme consists of a protein apoenzyme and a non-protein component Co-enzyme I, which is now known to be the very important compound diphosphopyridine nucleotide, or DPN for short. The catalysis of the hydrogenation of lactic acid occurs only when both fractions are attached to each other; they do not work independently.

Trace elements as activators

In the case of other enzymes the protein portion of the molecule has a simple *ion* attached to it. The ion can readily be removed, and the enzyme then loses its activity. If it is replaced the enzyme regains its catalytic properties. Thus, in enzymes like *tyrosinase* and *ascorbic acid oxidase* the copper ion functions in this way. As ions are chemically much simpler than the other prosthetic groups, it is common nowadays to refer to these ions as *activators* and not as co-enzymes. It seems likely that there are more trace-element *activators* than were previously suspected.

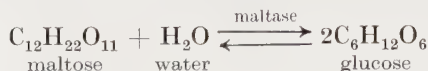
Not all trace elements are present as activators. They are also incorporated into the apoenzymes or are parts of prosthetic groups. It is because they are essential components of certain enzyme systems that trace elements play such an important role in biological processes, that deficiencies or excesses of them can seriously alter the enzymatic equilibrium of cells. Some of the enzyme systems affected will be discussed later in this chapter.

Enzyme reactions

Different enzymes carry out very different chemical reactions and there are an impressively large number of enzymes known. They are normally named after the substrate they catalyse, so that the name of an enzyme usually indicates its substrate or the nature of its activity. For instance, *urease* is the name of the enzyme that converts urea into ammonia and carbon dioxide.



Or *maltase* is the name of the enzyme which hydrolases maltose sugar to glucose sugar



This latter reaction also shows another characteristic of most enzyme systems, namely that the reactions they promote are reversible. So maltase can convert maltose into glucose, or glucose into maltose, depending on the environment.

Effect of temperature on enzymes

The influence of temperature on enzymes is very important. At very low temperatures, well below 0° C, most of them hardly function at all. In fact one of the experimental techniques to stop enzymatic reactions is to freeze the material being studied. On the other hand, heat inactivates and denatures enzymes. But between these two extremes there is a range of temperature, where heat greatly influences the rate of the reaction. There is a temperature, frequently between 30-40° C, at which maximum activity takes place. This is the *optimum temperature*, and is characteristic for any given enzyme system. Increasing the temperature above this point decreases activity, while lowering it below the optimal also decreases activity. Enzyme systems are very sensitive to temperature changes, and biological processes, such as digestion and respiration, show the similar temperature responses because they are controlled by enzymes.

Inactivation of enzyme systems

As well as being inactivated by heat, enzyme systems can be damaged by various poisons, especially heavy metals such as mercury, and they may be inhibited temporarily by a large number of chemicals, among them narcotics and anaesthetics.

All living matter is replaced continuously, as all living matter is in a dynamic state. There is always a turnover of proteins, fats, carbohydrates, as well as a slower replacement of structures such as bones, taking place in living organisms. Enzymes are no exception to this, and they are being broken down and replaced by new ones all the time, even before they are worn out. There is thus a continual need for the raw materials such as amino acids, from which new enzymes can be built. But some of this need is met by the breakdown products of the old molecules. However, not all this material is available, and so there is a *continuous* requirement by plants and animals for these raw materials. And in the case of enzyme systems containing trace elements there is obviously a continuous need for these.

Some important trace-element-containing enzyme systems

While there are some two hundred trace-element-containing enzyme systems known (or systems influenced by trace elements), very little is known about some of these, while others do not play a very important part in major processes. Therefore, only a limited number will be discussed because they are good examples of these systems, or because they have some particular importance from a biological point of view. The contents of Table 3 should make it clear that a detailed discussion of enzyme systems associated with trace elements is too extensive a topic for this chapter.

TABLE 3

List of enzymes known to be influenced by mineral elements. Five types of relationships are shown. From SCHWEIGART [270]

Column I. The trace element (or mineral element) constitutes the prosthetic group.

Column II. The trace element (or mineral element) is an active part of the prosthetic group, or is incorporated into the enzyme itself.

Column III. Elements with an integrating function that is not understood as yet. Elements need not be specific and may replace each other.

Column IV. Facultative Activators.

Column V. Inhibitors of enzyme activity.

ENZYME	I	II	III	IV	V
<i>Carbohydrases</i>					
α -Amylase (animal)	Cl		Na, K, Li, Br, Sr, Mg, Ca, Ba	F, I	
α -Amylase (malt)		Ca, Cl			Fe, Cu
Lysozym				S ₂ O ₃ , CN, S	I, Cu
Pectin-polygalacturonidase				K, Na, Rb, Ca, Th, Al	
β -D-Glucosidase			K	S	
Hyaluronidase				K, Na, Ca, Ba, Fe, Mn, Br, I, SO ₄ , NO ₃	F, Mg
<i>Esterases</i>					
Desoxyribonuclease			Mg, Mn, (Ca)	Ca, Ba	
Zoolipase			Ca, Mn, Cl	Pb, Mn, Na	
Phytolipase			Fe ⁺⁺⁺ , Al		F, PO ₄ , Ag, Cu, Hg ⁺⁺
Lipoproteinlipase				W, Mo, Si	
Pectase				I, CN	
Cholinesterase	Ca		Co, Ba, Mg, Cu, Mn	S	F
Acetylcholinesterase			NaCl		
Phosphatidcholinesterase			Mg		
Chlorophyllase			Ca	K, Na, Cl, NO ₃	
Alkaline phosphomonoesterase I		Zn, Co, Mn, Mg	Ni, Fe ⁺⁺ , Ca		SH
Alkaline phosphomonoesterase II				Mg	CN
Acid phosphomonoesterase I					F, Mg
Acid phosphomonoesterase II	Co		Ca, Mg, Co, Ni		

TABLE 3—continued

ENZYME	I	II	III	IV	V
Acid phosphomonoesterase III		Mg	Co	Ni	
Fructose-1,6-diphosphatase		Mg, Mn			Co, Ni, Zn, Cu
Phytase			Mg		
Nucleotidase			Mg		Zn, Fe ⁺⁺ , Ag, Cu ⁺⁺
Ribonuclease					F
Prostataphosphatase				Ce, La	
Tropinesterase			KCl, Na, Cl, NaBr, NaI, KCNS, MgSO ₄ , CaCl ₂		F, CN
Phosphoprotein-phosphatase					Mo
Phosphatidic acid phosphatase			Mg		Ca, Ba, Mg, Mn
Glycocyclaminase				Mn	
Arginindihydrolase				Fe	
Arylsulphatase				NO, Cl	
Desoxyribonuclease Type I (pancreas-nuclease)	Mg, Mn			Ca	F
Desoxyribonuclease Type II (urine nuclease)	Mg				F
Micrococcus nuclease	Ca				F
<i>Amidases</i>					
Defence proteinases	S			SH	Sch-Me
Proteinases in general			K		
Papainase I (papain)	S, CN, SO ₂			Ti, As, S ₂ O ₃ , SO ₂ , SH,	Sch-Me, H ₂ O ₂ , I, SeO ₂
Papainase II (papain)	S ₂ O ₄				
Cathepsinase	S, CN, SH, S ₂ O ₃ , SO ₃			Fe	Sch-Me (Hg, Cu)
Thrombokinas			Ca		
Bacterial endopeptidase	Ca				
Trypsinase			Ca, Mn, NH ₄ , MgSO ₄		
Rennin			Ca	Rare Earths	
Enterokinase			Ca		
Pepsinase			H, Cl		
Carosinase	Zn, Mn				PO ₄ , P ₂ O ₇ , F
Peptidase	Mg, Mn, Zn, Fe ⁺⁺ , Co ⁺⁺		Zn, Co		
Dehydropeptidase I					S, CN
Folic acid conjugase				S	Hg, Ca
Yeast polypeptidase				Zn, Cl, Br, I	
Aminotripeptidase			Me		
l-Leucin-amino-peptidase	Mg		Mn		CN, S, P ₂ O ₇ , Zn, Fe ⁺⁺ , Pb ⁺⁺ , Hg ⁺⁺
Aminopolypeptidase	Co, Zn		Zn, Co		
l-Cysteinyl-glycine-dipeptidase				Mn, Co, Fe	
Glycyl-l-leucyl-dipeptidase II				Mn	
Glycylglycine-dipeptidase	Co, Mn				Zn
Glycyl-l-leucine-dipeptidase I	Mn		Zn, PO ₄		Ca, (Zn · PO ₄)
Prolinase I	Mn, Cd				PO ₄ , P ₂ O ₇ , F
Prolinase II					S, CN, Ag
Prolidase	Mn				Sn, Hg ⁺⁺ , F, Pb ⁺⁺ , Cd ⁺⁺ , P ₂ O ₇

TABLE 3 *continued*

ENZYME	I	II	III	IV	V
Tryptic carboxy- polypeptidase	Zn	Mg			
Catheptic carboxy- polypeptidase			CN		
Aminopeptidase		Mn, Mg		Zn	
Histidase					Cu ⁺⁺ , Cd ⁺⁺ , Zn ⁺⁺ , KCN
DPN- and TPN- hydrolases				NO ₃ , NO ₂ , NH ₄	
Amino-acid acylases					Co
Arginindesimidase (yeast)		Co			Fe ⁺⁺ , I
Arginase a		Cd	Fe ⁺⁺ , Co, Ni		B, Zn, Mn
Arginase b		Mn	Cd, V	Ca, Fe, Ni	
Citrullinase		AsO ₃ , Mg, Mn, Co, Zn	PO ₄		
Asparaginase					Cu, Hg, Ag
Urease		Cd		S, CN, As, SO ₃	Sch-Me, F, B
Glycocarbaminase			Mn		
<i>Phosphohydrolases</i>					
Pryophosphatase I		Mg	Mn	Al, Mg, Zn, Zr, Th, Pb, Fe, Co, La, Ce, Y, CN	Ca
Pryophosphatase II		Mg	Mn	Al, Mg, Zn, Zr, Th, Pb, Fe, Co, La, Ce, Y, CN	F
Polyphosphatases			Mg		
ATP-ase I			Mg	Ca, Na	
ATP-ase II		Mg			K, Ca
Triphosphatase		Mg, Fe, Co			
Apyrase (ATP- diphosphatase)			Ca		
ATP-Pyro- phosphatase				Cl, Br	
Oligometa- phosphatase		Mg, Mn, Co, Zn		Ca, Ba, Al, Ti, Fe, Ni, Cu, Zr, Th, Pb, La, Ce, Pr, Nd, Sm, Y	CN, F
Polymeta- phosphatase			Mn, Mg, Zn, Ca, Pb		Ag, Hg ⁺⁺
Phosphohalogenase			Mn, Co		Hg ⁺⁺ , Zn, (Mn) Cu ⁺⁺ , Pb ⁺⁺
<i>Hydrolases with vary- ing substrate specificity</i>					
C-C-Hydrolases		B ₆ -PO ₄			
Halogenases				Cl, Br	
Iodine-tyrosine- deiodase				I	
Di-isopropylfluoro- phosphate halogenase			Ca, Mg, Co, Mn	Hg	Hg ⁺⁺ , Cu ⁺⁺
<i>Transglycosylases</i>					
Phosphorylase		Mg			
<i>Transphosphatases (Kinases)</i>					
Glucose-1- phosphate → amylose-trans- glucosidase				AsO ₄	
Saccharase → <i>ortho</i> -phosphate- transglucosidase				AsO ₄	

TABLE 3—continued

ENZYME	I	II	III	IV	V
Adenylate kinase			Mg		
Creatin kinase		Mg			
Arginine kinase		Mn	Mg		
1,3-Diphospho- glycerate $\rightarrow$ ADP-Trans- phosphatase		Mg	K, NH ₄		
Pyruvate kinase		K, Mg, NH ₄ , Rb			
Hexokinase		Mg			
Fructokinase		Mg	Mg, Mn	K	
Phosphofructokinase		Mg	K, NH ₄		
Galactokinase		Mg, Mn			
Phosphoglucokinase		Mg, Mn			
Gluconokinase		Mg			
Ribokinase (trans- ketolase)		Mg			
ATP $\rightarrow$ Nucleic- acid-trans- phosphatase		Mg, F	PO ₄		
Flavokinase			Mg, Mn		
Adenosinkinase			Mg, Mn		
Phosphoglucomutase			Mg		F
ATP $\rightarrow$ Nicotin amide-Mono- nucleotide-trans- adenylase		Mg			
ATP $\rightarrow$ FMN- Transadenylase		Mg			
ATP $\rightarrow$ DPN- Transphosphatase		Mg, Mn			
<i>Transaminases</i>					
Transaminase			Mg, NH ₄ , CO ₂		
Transglutaminase			Mn, PO ₄		
Transaspartase			Mn, PO ₄		
Carbamyltransferase		Mg			
<i>Transmethylases</i>					
Transmethylase			Mg, Ca		CN, F, Ca
<i>Transacylases</i>					
Acyl-activating enzyme (acetyl- Co-A)		Mg	K, NH ₄ , Rb		Na, Cs
Cholinacetylase			PO ₄ Ca, Mg, K		
<i>Special Transferases</i>					
Transketolase		Mg	PO ₄		
Rhodanase			S ₂ O ₃ , Cu		AsO ₃
<i>Anaerobic Trans- hydrogenases</i>					
Coenzyme III			PO ₄		
Codehydrogenase I = DPN			PO ₄		
Codehydrogenase II = TPM			PO ₄		
Aldehyde $\rightarrow$ TPN- transhydrogenase			PO ₄ , Ca, Ba, Mg, Mn		CN
Succinate dehydro- genase		Fe	PO ₄	Ca, Al, Co, Rare Earths	
Formiat $\rightarrow$ DPN- Transhydrogenase			PO ₄		
Formiat dehydro- genase		Cu	PO ₄		

TABLE 3—*continued*

ENZYME	I	II	III	IV	V
Oxalate dehydrogenase			Mg		
Choline dehydrogenase			Co		
Thiamine dehydrogenase				Mg	
α - β -unsaturated Acyl-CoA-reductase	Cu				
Fumarate reductase	Fe				
Saturase			Mn, Cl		
L-Dehydroascorbinate-reductase				PO ₄ , AsO ₄	
Lactate dehydrogenase	Fe				
Alcohol dehydrogenase	Zn				
Nitrate reductase	Mo		PO ₄	V	
Nitrite reductase	Fe		PO ₄		
Hyponitrite reductase	Cu		PO ₄		
Hydroxylamine reductase	Mn				
Gluthathion reductase			Mg, Mn, PO ₄		NaCl
<i>Aerobic transhydrogenases</i>					
Flavinenzyme I	Cu		PO ₄ , Mo, Fe		
Flavinenzyme II	Fe				
Diamine oxidase	Cu (?)		Co	PO ₄	CN, Ca
Xanthine oxidase	Mo, Fe		PO ₄		CN, Cu
Aldehydoxidase	Mo		NH ₄ , W		
Aldehydmutase (-oxydase)	Mo				
Bacterial Glucose-mannose-galactose-oxidase			Mg, Mn		
<i>Anaerobic trans-electronases</i>					
TPN-Cytochrome c-reductase	PO ₄				
DPN-Cytochrome c-reductase	Fe ⁺⁺ , PO ₄			CN	Cu, Zn, Mn, Ca, Mg, P ₂ O ₇ , V, PO ₄
Cytochrome a, b, c, f, a ₁ , a ₂ , b ₁	Fe ⁺⁺				
<i>Aerobic transelectronases</i>					
Cytochromoxidase (Warburg's iron ferment)	Fe		Cl		
Phenoloxidase	Cu	PO ₄ , Ni, CO, V	K, Mg, Ca, Zn, Mn, Al, Fe		
Ascorbic acid oxidase	Cu				CN
Laccase	Cu	PO ₄	Mn		
Urushiol oxidase	Cu				CN, S
Ceruloplasmin	Cu	Cu		Cl, Br, NO ₃	PO ₄ , SO ₄
Uricase	Cu	Mn			CN, F
Luciferase		Mg	PO ₄ , Mn		P ₂ O ₇
<i>Hydroperoxidases</i>					
Peroxidase	Fe ⁺⁺⁺		NO ₃ , I	Ce	

TABLE 3—continued

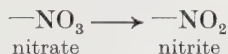
ENZYME	I	II	III	IV	V
Dioxymaleic acid oxidase		Mn, Fe ⁺⁺⁺		F	
Cytochrome c-Peroxidase		Fe ⁺⁺⁺			
Ferro-peroxidase		Fe ⁺⁺			
Lacto-peroxidase		Fe ⁺⁺⁺			
Myclo-peroxidase		Fe ⁺⁺⁺			
Indoyl-3-acetic acid peroxidase		Fe, Mn			
Catalase		Fe			
<i>Special redoxases</i>					
Lipoxydase				V, B, Fe	
Homogentisinase			Fe ⁺⁺		
Pyrocatechase			Fe ⁺⁺		
Lactonizing enzyme			Mn		
<i>Decarboxylases</i>					
Coccarboxylase		PO ₄ , Mg	Mn		
Plant carboxylase		Mg	Co, Zn		
Pyruvate-oxidase factor		PO ₄ , Mg			
Carboxylase (pyruvate decarboxylase)		Mg, PO ₄ , Mn		Co, Fe, Zn, Cu, Cd	
Benzoylformate decarboxylase		PO ₄			Mg
Oxylacetate-β-carboxylase		PO ₄ , Mn, Cd, Co	Mg		
Malic enzymes		Mn	Mg		
Malonate-decarboxylate system		PO ₄		Mg, Mn	
Succinate-decarboxylate system		PO ₄ , Mn			
Isocitric acid dehydrogenase		PO ₄ , Mn, Co	Mg		
Pyruvate dehydrogenase		Mg	Mn		
Bacterial amino-acid decarboxylases		PO ₄	Me, Fe ⁺⁺⁺ , Mn		
Yeast decarboxylase		Mg			
<i>Triosephosphate-lyases</i>					
Aldolase (cymohexase)		Mo	Zn, Fe ⁺⁺ , Co ⁺⁺ , Cu	B	
Asparatase	Mg		Co, Cu		
<i>Hydratases and dehydratases</i>					
Aconitase		Fe ⁺⁺			CN, S, F, Cu, Hg
Enolase	Mg		Mn, Zn	HPO ₄ , H ₂ , BO ₃	F, Hg
Carbo-anhydratase	Zn				CN, S
Chloroplastinase		Mg			
Phosphogluconate-dehydratases		Fe, Mn, Mg			
<i>Isomerases</i>					
Galactowaldenase		PO ₄			
D-Arabinose-isomerase				B, OH	
Glucose-1,6-phosphomutase			Mg, Mn, Co		
Glycerine-3,2-phosphomutase			Mg		
<i>Racemases</i>					
Alanine racemase			PO ₄		

TABLE 3—*continued*

ENZYME	I	II	III	IV	V
Glutamate racemase				PO ₄	
<i>C-N- and C-S-lyases and synthases</i>					
Cystein- desulphydrase			Zn, Mn, Mg		
<i>Unclassified lyases and synthases</i>					
Hydrogenlyase		Fe	Mn, PO ₄		
Hydrogenase		Mo	PO ₄		

Nitrate reductase

Molybdenum is a trace element required in minute quantities. In Australia, where molybdenum deficiency is very pronounced in some areas, only 2 oz. of molybdenum per acre (138 g. per hectare) are required to make good this deficiency and restore normal growth of vegetation. The role of this element has been widely studied, and Nicholas and Nason [211] demonstrated that one of its most important functions in plants, although not its only one, was to be a constituent of the enzyme *nitrate reductase*. This enzyme converts nitrates to nitrites.



This is an important enzyme because plants normally absorb their nitrogen from the soil in the form of nitrates. In agriculture nitrogenous fertilizers and organic manures are added to the soil, where bacteria convert much of the nitrogenous matter into nitrates. The plants absorb the nitrate, and this must then be converted into re-

TABLE 4

Nitrate content of normal and molybdenum-deficient cantaloupe leaves grown on sandy soil. From EVANS [98]

DESCRIPTION OF LEAVES	NITRATE NITROGEN IN DRY LEAVES, P.P.M.
Normal	900
Deficient	5,880
Normal	743
Deficient	3,965
Deficient*	1,040

* Sprayed with a 0.1% Na₂MoO₄ solution before harvesting leaves.

The deficient leaves show appreciably higher levels of nitrate than the normal ones. Even spraying the leaves with a solution of a molybdenum salt enables the plant to obtain molybdenum for its nitrate reductase, as the last figure clearly shows. Its level of nitrate has returned to almost normal.

duced nitrogen, i.e. -NH_2 before it can be used to synthesize amino acids, which are used to build up proteins. The reduction of nitrate to nitrite is catalysed by nitrate reductase and is the first stage in this reduction. If there is not enough molybdenum available to the plant, then this enzyme cannot be manufactured in sufficient quantity, and the nitrates that are absorbed cannot be reduced, and hence they accumulate. Table 4 shows the accumulation of nitrates in deficient cantaloupe leaves. In some cases the level of nitrate may rise to such levels that it causes nitrate poisoning in stock (an example of an indirect toxic effect).

It is clear that molybdenum deficiency can seriously upset the nitrogen metabolism of the plant. If it is acute, clearly recognizable deficiency symptoms set in. In many plants, if the soil nitrate is replaced by other reduced forms of nitrogenous material, such as ammonium salts or nitrites, the need for molybdenum is not nearly so acute, and the plant continues protein syntheses fairly normally

TABLE 5

The influence of the presence and absence of certain elements in the substrate upon the growth and nitrate reductase activity of the fungus Neurospora crassa (Wild type 146). From NICHOLAS [210]

ELEMENT	GROWTH, % OF NORMAL MYCELIUM	NITRATE REDUCTASE ACTIVITY†
+Ca	100	16
-Ca	26	38
+Mg*	100	49
-Mg	20	41
+Fe	100	29
-Fe	0.7	55
+Cu	100	27
-Cu	40	79
+Zn	100	25
-Zn	28	39
+Mn	100	30
-Mn	30	34
+Mo	100	53
-Mo	29	10

* $\frac{1}{100}$ the concentration of the control.

† Units of enzyme activity expressed as $\text{m}\mu$ moles NO_2 formed per 10 minutes per mg. protein.

+ = present. Fungus grown on the complete substrate.

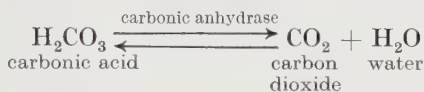
- = absent. Fungus grown on a substrate lacking only this element.

[132 and 133]. The plants still require molybdenum for other enzyme systems, but in much lower concentrations. It is clear that nitrate-nitrogen increases a plant's requirement for molybdenum.

Lower organisms, such as fungi, require molybdenum for their nitrate reductase just as green plants do. As Table 5 clearly shows, molybdenum is the only element whose deficiency results in a marked decrease in this enzyme.

Carbonic anhydrase

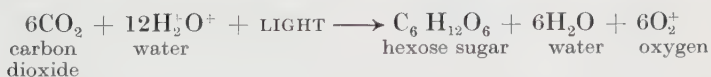
Carbonic anhydrase is a very important enzyme with *zinc* in its prosthetic group. It is found in animals and plants. It catalyses the splitting of carbonic acid into water and carbon dioxide, and vice versa.



The studies of Henriques [127] showed quite clearly that the rate at which carbon dioxide was removed from the blood and liberated by the lungs required an enzyme to promote it. Subsequently, this enzyme was isolated from the erythrocytes or red cells of cow's blood [187], and since then carbonic anhydrase has been found to be widespread, and the fact that zinc is a constituent confirmed.

This enzyme is responsible for the rapid removal of carbon dioxide from the blood. In its absence the carbon dioxide would accumulate and would slow up the entire respiratory process, because of the accumulation of 'waste' products. In the absence of this enzyme a much larger living surface would be required to remove the carbon dioxide from the blood.

In plants, where rates of respiration are lower than in animals, the problem of the disposal of carbon dioxide is not so acute. But green plants need an efficient system to absorb carbon dioxide from the air in order to carry out *photosynthesis*, this fundamental process whereby the plant converts inorganic matter, such as carbon dioxide and water, into organic matter, such as sugars, with the aid of sunlight. This is the way in which organic matter is primarily synthesized.



But carbon dioxide is present in very small amounts in the air; usually only about 0.03 per cent. So that while the leaf is an efficient absorbing organ for this gas, the catalytic conversion of it into carbonic acid is necessary if the carbon dioxide is to be removed rapidly and used at the observed rates in photosynthesis. Because of experimental difficulties, and especially as zinc is a component of

other important enzyme systems, it has not yet been possible to show directly that zinc deficiency brings about a marked reduction in photosynthesis due to lack of this enzyme. This is not really unexpected, as biological reactions are frequently extremely complex.

But experimental data show quite clearly that a zinc deficiency results in decreased carbonic anhydrase activity. An example of this is shown in Table 6, which shows the levels of carbonic anhydrase

TABLE 6

Variation in the carbonic anhydrase activity of normal and zinc deficient oat leaves, at different ages and zinc levels in the leaves. After WOOD & SIBLEY [332]

NO. OF DAYS AFTER SOWING	CARBONIC ANHYDRASE ACTIVITY, ENZYME UNITS PER GR. DRY MATTER		ZINC CONTENT, IN P.P.M. DRY MATTER	
	ZINC		ZINC	
	CONTROL	DEFICIENT	CONTROL	DEFICIENT
21	104	104	76	76
49	111	110	276	87
91	120	80	119	27
121	81	41	41	11
140	50	34	19	7

Note that the young leaves have adequate supplies of zinc, even if grown on a zinc-deficient substrate. These are derived from reserves in the seeds. It is in the older leaves in which zinc deficiency is observed, as is a decrease in carbonic anhydrase activity.

activity in normal and zinc-deficient oat plants. The general picture of zinc deficiency being coupled with decreased carbonic anhydrase activity is quite clear.

Xanthine oxidase

An enzyme of great importance in animals is *xanthine oxidase*, a molybdenum-containing oxidase which is responsible for the final stages in the formation of uric acid, a waste product that is eliminated via the kidneys in the urine. It is particularly plentiful in the livers and intestines of many animals, and its concentration is very closely linked to the amount of molybdenum in the diet, as is clearly shown in Table 7.

The enzyme promotes the conversion of hypoxanthine $\rightarrow$ xanthine $\rightarrow$ uric acid. In parts of New Zealand where soil molybdenum was very low serious sheep losses were experienced due to stones in the kidneys of the sheep. Analysis of these renal calculi showed that they were pure xanthine. In the near absence of molybdenum, and hence inadequate supplies of xanthine oxidase, the normal conversion of

TABLE 7

The influence of dietary molybdenum on the molybdenum content of the liver, and xanthine oxidase activity in rats. From COX et al. [72]

Experiment No. 1	MOLYBDENUM LEVEL IN DIET, P.P.M.	LIVER XANTHINE OXIDASE*	LIVER MOLYBDENUM†
	0	227.5 ± 38.6	14.7 ± 5.1
	500	151.0 ± 76.4	24.1 ± 7.3
	800	119.4 ± 94.9	25.9 ± 5.5
Experiment No. 2	0	190.1 ± 25.0	11.6 ± 5.7
	250	181.8 ± 35.2	18.3 ± 3.2
	800	157.6 ± 27.0	25.8 ± 2.8

It is clear from these figures that there is a relationship between dietary molybdenum and the level of this element in organs such as the liver. But the levels of molybdenum used were so high that clinical signs of toxicity were produced and this excess of molybdenum decreases the liver xanthine oxydase activity. The element that activates an enzyme may be harmful in excess.

* Measured as $\mu\text{l. O}_2/\text{g.}$ of dry homogenate 20 minutes.

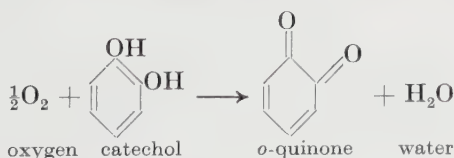
† Measured as $\mu\text{g.}/\text{g.}$ of dry homogenate.

xanthine into uric acid was hindered and the xanthine was precipitated in the kidneys instead of eliminated, with fatal results [29].

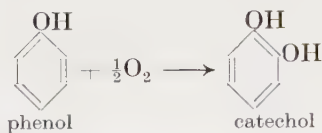
Oxidases

The oxidases are a group of enzymes which promote oxidative processes, of which there are a large number in living matter. They transfer hydrogen or electrons directly to oxygen as a rule, and are of great importance, especially in respiratory processes, where they are responsible for the ultimate oxidation of the organic matter being broken down. Of these the cytochrome oxidases, many of which contain *iron* in their prosthetic groups, have been known for a long time. They are responsible for the terminal oxidation of most of the organic matter in many organisms. The prosthetic group, a haeme, is very similar to part of the red pigment in blood, haemoglobin. Copper-containing oxidases are nearly as important as the ones containing iron.

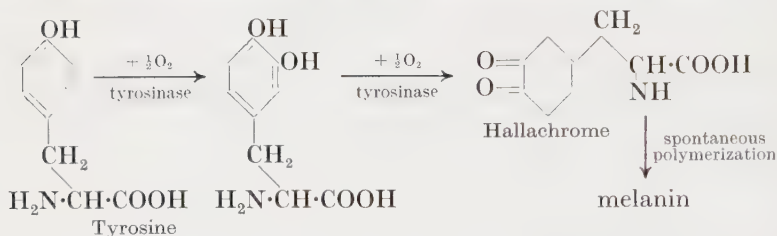
Polyphenol oxidase, *laccase*, and *ascorbic acid oxidase* all contain copper and cannot function without it. A polyphenol oxidase, called *tyrosinase* is a good example of a direct oxidase. It promotes the conversion of catechol to *ortho*-quinone by direct oxidation with oxygen.



As well as this, the same enzyme will oxidize a monohydric phenol to a dihydric one, the new group being inserted into the *ortho* position as follows:



Because of this ability it can bring about the oxidation of the amino acid tyrosine to give rise to the dark-coloured pigment melanin.

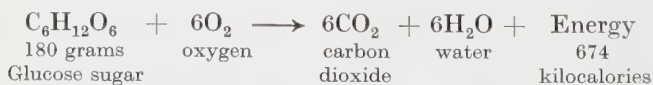


This is a relatively simple demonstration of the ability that some enzymes have of catalyzing a number of different reactions on various substrates. Not all enzymes are capable of doing this.

RESPIRATORY PROCESSES AND TRACE ELEMENTS

Respiration

Respiration is an enzymatic process taking place in *all* living matter, and it is the progressive oxidation (or slow combustion) of energy-containing substances such as carbohydrates. This oxidation takes place in a large number of stages and ultimately releases *energy*, which is utilized by protoplasm. As all living cells require a continuous supply of energy to remain alive, it is clear that this process is one of the most fundamental in biology. The equation for the overall reaction in the aerobic respiration of hexose sugar shows the nature of this process very clearly.



All the energy in the sugar has been liberated as a result of the complete oxidation of both the carbon and the hydrogen. Not all cells can carry out this oxidation so effectively as the equation indicates.

Certainly, a study of the gas exchange of tissues, organs, or entire organisms gives much insight into respiratory activities, and the influence of trace elements on respiration is very spectacular, even if

it cannot always be explained in biochemical terms. Lundegårdt [177] found that increasing the *manganese* content of the solutions in which he was growing wheat increased the oxygen consumption of the roots by several hundred per cent. On the other hand, *zinc* deficiency in the mould *Aspergillus niger* increased its respiration rate above normal [200]. *Boron* has very contradictory effects upon the respiration rate of plants, and this can perhaps be best explained by assuming that only when sugar is the limiting factor in respiration do increases in boron increase the respiratory rate. Boron can regulate the availability of sugar, cf. page 29 [111].

Phosphorous compounds in respiration

In respiration the main energy transfers are carried out through various organic phosphorous compounds, especially *adenosine triphosphate* (ATP), *adenosine diphosphate* (ADP), and *diphosphopyridine nucleotide* (DPN).

The compounds are influenced by molybdenum, selenium, chromium, and tungsten, and *in vitro* they can play a very important part in determining their reactions, but their role in these systems *in vivo* is not clear [331]. It is quite certain that zinc deficiency in the mould *Neurospora* leads to a 10–20 fold increase in the concentration of the enzyme *diphosphopyridine nuclease* (DPN ase) [204].

TABLE 8

Loss of liver cytochrome oxidase activity in rats on a copper-deficient diet.

From GALLAGHER et al. [106]

STAGE OF DEFICIENCY	TIME ON DIET, WEEKS	LIVER COPPER μg./G. DRY WEIGHT		LOSS OF ACTIVITY: COPPER DEFICIENCY, AS %
		DEFICIENT	CONTROL	
Very slight	4	8.5	19.3	76
Moderate	10–11	3.1	14.8	85
Extreme	13+	0.9	14.3	96

It is of interest that the cytochrome oxidase activity has dropped by as much as 76 per cent after only one month on a copper-deficient diet, while the liver concentration is still half that of the control, and the deficiency is still only slight.

Gallagher [105],* in his detailed investigations on rats, found that acute deficiency of copper resulted in reduced DPN-cytochrome reductase. The mitochondria in the cells of the deficient rats were found to be very low in pyridine nucleotides.

The most important consequence of copper deficiency in the rats was a marked decrease in liver *cytochrome oxidase*, a copper-containing

* See C. H. Gallagher, *Enzymological Disturbances in Animals*, in this series of monographs.

enzyme important in the terminal oxidations of respiration. Table 8 shows how the decrease in liver copper, due to a deficient diet, is linked with marked reductions in enzyme activity. This is reversible, and the addition of copper to the diet results in increased liver copper and *cytochrome oxidase* activity. In extreme copper deficiency there is a rapid loss in body weight in the moribund rats, because the liver enzymes are incapable of oxidizing any substrate.

In the respiration of sugar the Krebs or tricarboxylic acid cycle

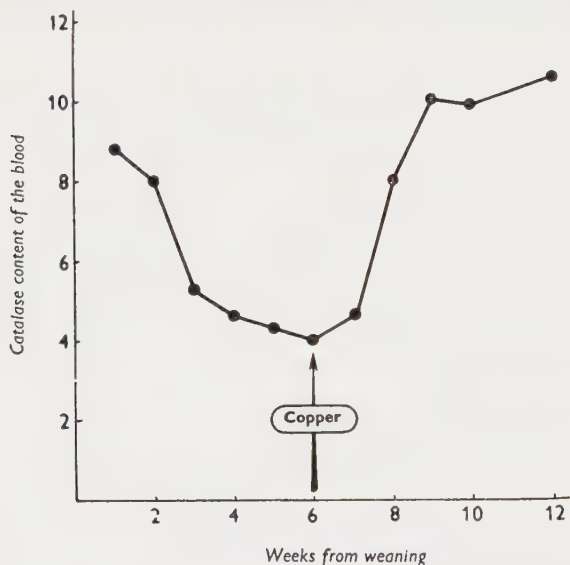


FIG. 4

FROM SCHULTZE [260]

The influence of copper deficiency on catalase activity in the blood of rats

The rats, which were raised on a copper-deficient diet until six weeks after weaning, were given a copper supplement of 0.1 mg. Cu/day, from which time the catalase activity rose rapidly.

is also influenced by trace elements, iron and manganese playing a part, but the most important mineral element undoubtedly being magnesium [202].

The ubiquitous iron-containing enzyme *catalase*, which destroys hydrogen peroxide in the cell and also oxidizes many other substances, is of great interest. It contains haeme as a prosthetic group, as does cytochrome oxidase, the same as the haeme as is found in the haemoglobin of blood. Copper plays a very important part in the synthesis of haeme in animals, and thus copper deficiency can result in a decrease in catalase activity. This can be overcome by supplying copper in the diet, as can be seen diagrammatically in Fig. 4. This

shows the rapid increase in catalase activity in the blood of deficient rats when copper was supplied in the diet [260]. Not all workers get these same results, for Gallagher, in the work referred to above, found *no* decrease in catalase activity in copper-deficient rats, although he found that haeme and cytochrome oxidase were markedly depleted. The varying experimental conditions probably cause this contradiction. Certainly, in plants catalase activity can be directly related to available iron [4], as is clearly shown in Fig. 5 in the case of sun-

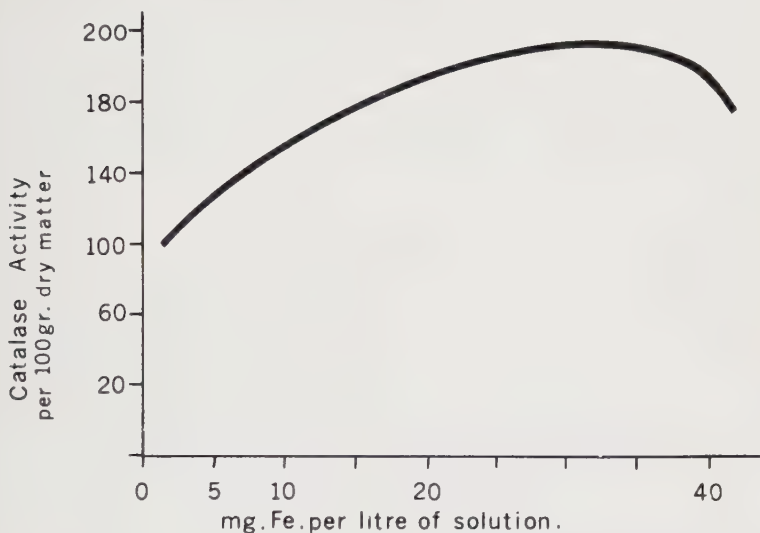


FIG. 5

FROM AMBERGER [4]

The influence of deficiency and excess of iron in the nutrient solution on the activity of catalase in sunflowers grown in water culture

Iron citrate used as a source of iron

The activity of the catalase increases with increased iron, until an optimum activity is reached. Any further increases in iron concentration result in a *decrease* in activity.

flowers, and it is worth observing that after an optimum iron concentration has been reached any increases result in decreased activity of the enzyme.

Respiratory pigments

In larger organisms the process of respiration is complicated by the fact that a suitable mechanism must exist to make oxygen available to the living cells of all the tissues. In animals and man this is accomplished by means of respiratory pigments which are circulated in the blood. And many and perhaps all of those contain trace elements.

These pigments are complex proteins which transport oxygen from organs such as the lungs to the body tissues. *Haemoglobin*, the red colouring matter in mammalian blood, is one of these iron-containing pigments. The non-protein part of the molecule is a porphyrin, with a very characteristic chemical structure. This porphyrin structure is found in haeme, as well as in some important enzymes such as catalase and the cytochromes, all of which contain iron-porphyrin prosthetic groups attached to their proteins. Myoglobin, the oxygen-combining protein of muscle, has a similar structure.

Other respiratory pigments are found in invertebrates. *Haemocyanin*, which is found in the blood of crabs, squids, and octopuses, is a respiratory pigment with copper in its prosthetic group. Sea squirts contain vanadium. Marine worms like *Phaseolus*, have an iron-containing pigment, haemerythrin. This reddish compound is quite different from those mentioned previously, as it does not contain a porphyrin.

End products of fermentations

Fermentations give rise to a large number of products, but trace elements can play an important part in altering the nature of these. Normal cultures of *Clostridium welchii* produce acetate, butyrate, and ethyl alcohol when they ferment glucose; but in the absence of iron they produce mainly lactic acid [200]. Zinc-deficient cultures of *Rhizopus* accumulate fumaric acid and of *Aspergillus niger* citric acid. Very clearly, trace elements play an important part not only in normal aerobic respiration but also in these various fermentations, which are controlled by such a variety of enzyme systems.

Non-enzymatic catalysis by trace-element ions

So far, the part played by trace elements in enzyme systems has been discussed. But while this is undoubtedly the most important part, these elements also function in other ways. For instance, metal ions can function as catalysts on their own, without being associated with enzyme systems.

Krebs [160] showed that aluminium (Al^{+++}), iron (Fe^{++} and Fe^{+++}), and copper (Cu^{++}) are active in decarboxylating oxaloacetic acid, an important constituent of the respiratory cycle. Other workers [156] reported similar reactions in the decarboxylation of keto and other organic acids. Metallic ions are quite capable of catalysing reactions in organic systems, and they are definitely known to exist in living tissue. But there is virtually no information available to show to what extent these catalytic reactions take place in living cells.

There is no doubt that the catalytic properties of metallic ions are remarkably enhanced and made more specific if they are bound to certain proteins, as occurs in the metallo-enzymes. The catalysis

of the oxidation of ascorbic acid by copper ions is enhanced 1,000 fold when the copper is incorporated into the enzyme *ascorbic acid oxidase* [185], but they can and do catalyse this reaction on their own.

Another quite different type of catalysis is found in the case of boron in plants: to date this element has not been shown to be a constituent of any enzyme complex, although it can function as an inhibitor. Yet its influence upon plant development is very great [111]. It is necessary for the efficient translocation of sugars around the plant, and in boron-deficient plants sugars tend to accumulate in the leaves. It is known to play a part in both fat and carbohydrate metabolism, yet how it does so is not known. Nor is the way in which it promotes the uptake of cations (positively charged ions). Yet Rehm [236], in his studies on the nutrition of the garden balsam, *Impatiens balsamina*, found that as little as 0.5 mg. boron per litre of culture solution increased the uptake of potassium, a major nutrient, 21 fold over plants growing in solutions lacking boron. When 10 mg. boron were supplied, the potassium level rose to 95 times that of the controls (cf. Chapter XII for boron: potassium interactions). These results must certainly be considered as catalytic, although their nature is unknown.

The significance of trace elements in enzyme systems

The entire physiology of the organisms is dependent upon the biochemical changes that occur within the cells. The vast number of chemical reactions which take place in these microscopic cells are regulated by the 2,000 or more different enzymes present in each of these cells. But many of these enzymes are greatly influenced by trace elements, especially the metallic ones. Kench [150], in a discussion of the parts played by metal ions in living cells, stresses that they form what he calls a 'cationic climate', in which interactions take place. He clearly indicates in enzymatic processes the complex competition for sites on enzymes and substrates which takes place at sub-cellular level, and how a preferential absorption of certain trace elements can either block or promote enzyme activity, and hence alter the entire biochemistry of the cell. The number of ways in which they can influence the cell's metabolism are numerous, and the complexity of investigations at this level can best be visualized by Kench's own words: 'Around all the macro-molecular structures in the living cell hovers a cloud of metal ions, jostling for position on the surface of the large molecules and according to their numbers and characters, in the case of enzymes, helping or hindering the movement of molecules substrates or products—to and fro.'

'The wealth of living forms is reflected at the cellular and sub-cellular level by a vast number of possible molecular inter-relationships, among which the relatively indestructible metal ions appear to

have been exploited fully in a directive capacity, quickening or slowing the rate of structural change of the more evanescent carbon compounds, helping to provide these metabolic bridges and feeds-back'.

It is with this background in mind that the other aspects of the biology of trace elements must be studied, even if the chain of events linking the sub-cellular phenomena with the activities of co-ordinated animals and plants has not yet been forged.

III | Deficiency and excess

THE INTERACTIONS OF TRACE ELEMENTS AND THE CONSEQUENCES OF UNBALANCED MINERAL NUTRITION

From what has been discussed so far, it is clear that an *imbalance* of mineral nutrients can occur in plants or animals. But it is possible that in practice living matter is so adaptable to its environment that deficiency or excess of these elements does not result in clinical symptoms, and so no practical problems connected with these exist. However, this is not the case. Well-established trace-element deficiencies are known, and they do constitute real practical as well as theoretical problems, as will be shown in the rest of this book.

Historical background

It must be stressed that trace-element deficiencies are widespread, and have been known from the earliest historic times. Their causes were not understood, but the conditions were recognized, and in some cases successfully treated.

For instance, simple iron deficiency anaemia was known in Roman times, and was treated with dilute iron salt solutions, as it is today. Many tonics which are given to cure anaemia and various forms of lassitude make use of dilute iron salt solutions, containing other trace elements, such as copper and manganese, which also influence haemoglobin formation. Simple iron-deficiency anaemia is still a serious health problem, and is very widespread, cf. Chapter IX.

Simple goitre is so widespread that in many parts of the world the slight swelling at the base of the throat, which is found in mild cases, is not even recognized as abnormal, cf. Fig. 6. It is also very widespread in animals (Fig. 44, page 98) too. The vast extent of its distribution is shown clearly in the world goitre map (Fig. 55, page 133). Yet 2,000 years ago the Greeks treated this condition quite successfully by giving sufferers the ash of sponges to eat. This material is very rich in iodine, and so helped to cure the goitre, which is an iodine-deficiency disease. Thus, a knowledge of modern chemistry was not necessary therapeutically to treat this condition. In fact, it was only within the last century that goitre was recognized to be a disturbance of iodine metabolism.

Scattered throughout the world are areas which will not allow

stock to develop normally. In England 'teart' pastures have been known for centuries. On these pastures, which look normal, cattle and sheep develop 'scours' (diarrhoea), rapidly lose weight and condition, and if left to graze on these swards die. Yet horses can thrive on them. What is the cause of the 'scours'? Today we know that it is due to too high a level of molybdenum in the vegetation, which induces a relative copper deficiency. Cattle and sheep have a marked



FIG. 6

FROM PÉREZ, SCRIMSHAW, AND MUÑOZ [220]

Goitre, a condition caused by disturbed iodine metabolism

Goitre results in the thyroid gland of the neck swelling and, in general, the size of the thyroid is a good index of the severity of the goitre.

The boy shows a mild goitre, in which the swelling of the gland is only obvious when the head is tilted backwards.

The goitre in this woman is quite obvious, and the 'big neck' can be seen from a considerable distance. This is a not uncommon size for a goitrous gland, but in unusually severe cases glands of several pounds weight have been recorded.

copper requirement, while that of horses is much lower. The molybdenum in the grazing prevents the cattle utilizing the copper present. It *antagonizes* it. The 'scours' can be cured by giving the animals extra copper, and when their copper requirements are met they can thrive on such pastures.

In parts of the mid-west of the U.S.A. large areas are quite unsuited for raising stock because of a chronic selenium poisoning termed 'alkali disease'. Fig. 7 shows a photograph of the abnormal develop-

ment of feet in cattle that takes place in this condition. The animals hobble around in great pain and eventually die.

Just as these trace-element deficiencies and excesses are known in man and the animals, so they are well known in plants as well. 'Brown rot' in swedes, and 'heart rot' in sugar beet are caused by boron deficiency; 'little leaf' of citrus and deciduous fruit is a zinc



*Photo by courtesy College of Agriculture and Experiment Station,
The University of Wyoming, U.S.A.*

FIG. 7

The fore-feet of a cow suffering from 'alkali disease', a form of chronic selenium poisoning

The effect is not so severe in Wyoming as is sometimes seen in South Dakota, possibly because the sources of selenium in the animal diet are mainly the native range plant, rather than cultivated grain. In more severe cases a separation of the hoof at the coronary bend is quite evident.

deficiency; 'gray speck' of oats and 'marsh spot' of beans are due to inadequate supplies of available manganese; and 'whip tail' (Fig. 8) in cauliflower is a molybdenum deficiency. These and a host of other deficiency diseases have been known for generations, although it is only within the last thirty years that the causes of these conditions have been identified. They are true nutritional disturbances, and are

not caused by pathological infections of unidentified fungi or virus. As more of these conditions are being identified, so the increasing extent of these nutritional disturbances and their implications is being appreciated.

The fact that a plant's nutrition is so intimately associated with the soil upon which it grows, makes it easier to observe deficiencies in them than in animals. In Europe 'heart rot' in sugar beet is very widespread on certain types of sandy soils. As is clear from Fig. 9.



Courtesy Messrs E. J. Hewitt and E. W. Jones, Long Ashton Research Station

FIG. 8

Molybdenum deficiency in cauliflower

Molybdenum deficiency results in this characteristic condition termed 'whip-tail'. The normal broad lamina of the leaf is not formed and the mid ribs remain with shreds of leaf blade attached. This type of plant is of no commercial value.

the boron-deficient plants are much smaller in size than normal ones, and much of the roots has rotted away and is usually infected by a parasitic fungus. This obviously represents a very great economic loss to the farmer. Brandenburg's [46] classical researches in 1938 clearly showed that 'heart rot' was caused by boron deficiency, and that the fungal infection usually associated with this condition was only a consequence and not a cause of it. Applications of borates completely cured this condition.

The new era in trace-element nutrition

Brandenburg's work did more than just show the cause of 'heart rot' in sugar beet. It effectively ushered in a new era to agriculture. Before his time, other scientists had appreciated the significance of trace elements in the health and well-being of plants and animals. Medical men had been using iodine and iron in their therapy for a long time. All these activities plus the more accurate chemical and biological techniques that had been developed gave rise to a climate of opinion in which the significance of Brandenburg's work could be properly appreciated and its wider importance assessed.



Borax Consolidated Ltd, Photo J. Dunlop

FIG. 9

'Heart rot' of sugar beet, due to boron deficiency

'Heart rot' is a very widespread type of boron deficiency found in sugar beet. Not only is the deficient plant stunted, but the crown rots away. In this picture secondary crowns have developed in the deficient plants on the right. Deficient plants are very frequently infected by the fungus *Phoma beta*.

Today it is generally recognized that minute quantities of certain elements can play an important part in health and disease, and the importance of trace elements is known to quite untrained agricultural workers. It is common for daily newspapers to discuss such subjects as the fluorination of municipal water supplies, when it is proposed to add minute quantities of fluorine to the water in an effort to combat dental caries in urban populations. And in the Press there is much opposition to such measures, as fluorine is known to be such a toxic substance, and fluorine toxicity is recorded in all five continents.

In the last thirty years great strides have been made in the scientific studies of trace-element nutrition and in the practical application of these findings to agriculture and medicine.

Variability of requirement

One of the important points that has been clarified as a result of research is the fact that the mineral requirements of different forms of life vary very considerably. It has already been pointed out that horses will flourish on 'teart' lands, where cattle and sheep pine and die. It is certainly true that domestic ruminants have a higher copper requirement than horses, but it is not true of all ruminants. In Africa, where extensive areas of copper-deficient pastures are found, as on the Kwango Plateau of the Republic of the Congo, on which domestic cattle die, buck and local wild ruminants thrive. They are obviously adapted to these conditions, or they would not have survived. Adapted vegetation is also found. This adaptation is a phenomenon to which a few words must be devoted.

Adaptation to unbalanced nutritional conditions

In plants, as in animals, there is a very wide range of requirements even between closely related forms (see Chapter X). The manganese requirements of oats are higher than those for either barley or wheat, and so 'gray speck' in oats, a manganese deficiency, is well known, while its equivalent in the others is not nearly so common. Wild cereals flourish where domestic ones cannot survive. As Marston [180] writes about copper deficiency: 'Some species of plants . . . are better able to derive their copper requirements from penurious soils, for a copper deficient terrain almost always has an indigenous (i.e. adapted) flora.'

This is certainly true of the copper-deficient North German Plain, a region stretching from Holland to Russia [230]. In this region birch, bog-wheat, and other adapted plants flourish. Rademacher [229] studied how locally developed varieties of oats could grow normally in these regions, while imported high-yielding varieties could not thrive under these conditions. He showed that in oats adaption did not consist in a decreased copper requirement, but in an ability to absorb copper early in the growing season. The imported varieties absorbed copper slowly throughout their growth, and suffered from deficiencies, as copper is not readily available from dry soils, and the soils dried out during the summer.

This ability to absorb copper rapidly is obviously genetically determined. Studies of grasses adapted to grow on ore dumps shows that some members of a given species may be 'tolerant' to very high levels of lead, zinc, nickel, etc., in the soil, while other plants of the same species just cannot develop normally on these poisoned soils [45, 143].

Certainly differences in requirement also exist, and in Florida the grass *Setaria* not only flourishes but actually accumulates zinc on

soils where the cerea maize shows acute zinc deficiency [262]. Another grass well adapted to deficiency conditions is St. Augustine grass *Stenotaphorum secundatum* (Salt.) Kunze, which has a very low copper requirement [82]. The fact that certain plants have a marked requirement for a certain element, such as cauliflower has for molybdenum, makes them useful as plant indicators of soil conditions, cf. Chapter X.

Interaction of elements

The fact that deficiencies and excesses of elements occur might lead one to conclude that an absolutely fixed minimum and maximum level for any element might be established in a given organism.

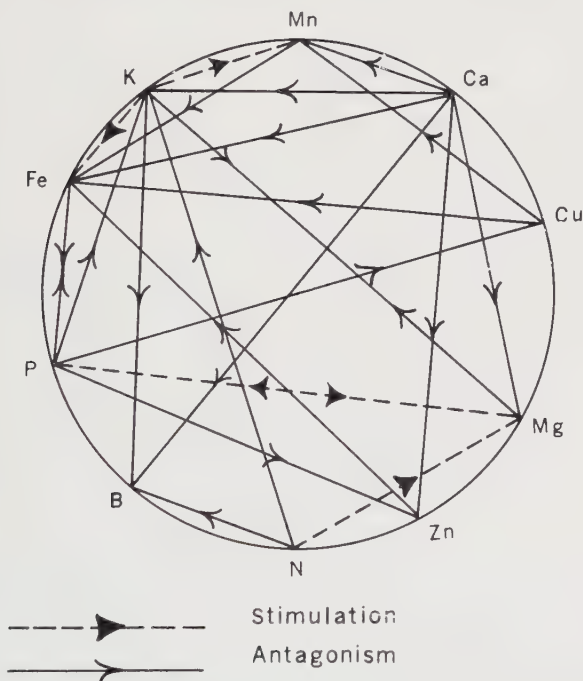


FIG. 10

AFTER MULDER [198]

Mulder's Interaction Chart

Every element in a nutrient solution influences the others, and a *balance* must be maintained between all nutrients, if healthy, efficient growth is to take place. Mulder's Interaction Chart shows this relationship between the elements very clearly.

While this chart is very valuable, it must be remembered that it is a generalization. All plants vary to some extent in their mineral requirements, and so this chart does not depict the responses of every plant species.

Unfortunately, this is not the case, because an organism's requirements are influenced by a host of factors besides its genetical constitution. Temperature, rainfall, daylength, drainage, and such factors all play a part, but the most important determining influence is the nature of the other nutrients, especially the other mineral nutrients.

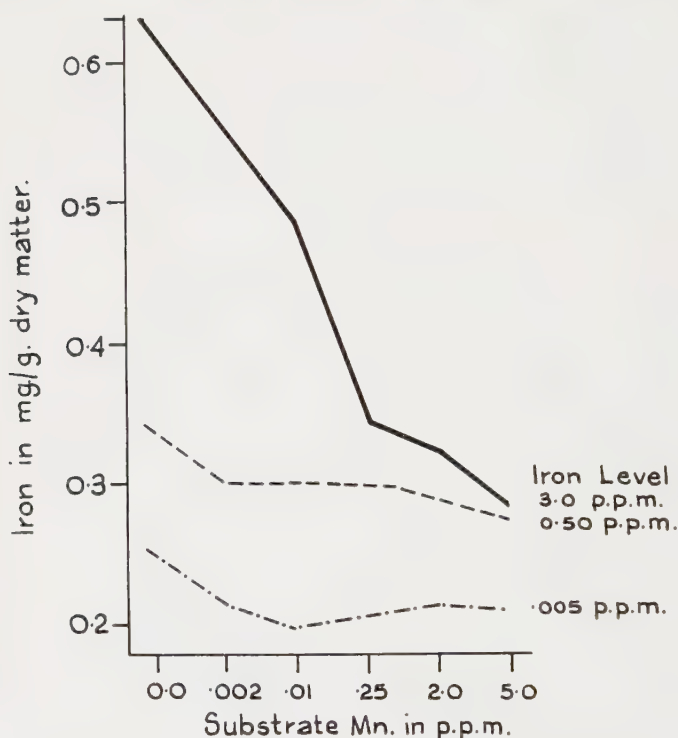


FIG. 11

AFTER SOMERS AND SHIVE [281]

The total iron content of soyabean plants grown at three levels of iron, plotted against the manganese concentration in the substrate

It is quite obvious that increasing concentrations of manganese depress the iron content of the soyabean plants. The higher the substrate level of the iron, the more marked is the depression. There is a clear-cut antagonism between the iron and manganese, which can influence the mineral composition of the plant to a very considerable extent.

An iron: manganese ratio of about 2.0 is found in normal plants.

All the mineral nutrients interact and influence each other. This is shown very clearly in Mulder's Interaction Chart (Fig. 10), which depicts typical responses in plants [198]. Thus, high levels of phosphorus in the soil will *antagonize* both copper and zinc uptake or use. The plant will require larger quantities of these nutrients if it is to

remain normal than would a similar one in a soil low in phosphate. In fact, the use of *excessive* phosphate on normal soils may result in *induced* zinc deficiency, i.e. a deficiency resulting from too little zinc for this level of phosphorus. Induced deficiencies are widespread and constitute a serious agricultural problem. Some of these are discussed in detail in Chapter XII. Other antagonisms are also well known, and the interaction of iron and manganese is one that has been studied extensively. This antagonism is clearly depicted in Fig. 11.

This same state of affairs exists in animals, and reference has already been made to the increased copper requirements of cattle when they are grazed on pastures rich in molybdenum.

For normal development, a *balanced* nutrition is essential. If there is too little or too much of any given nutrient, then this balance is upset, and either deficiency or toxicity symptoms of some sort will develop, for these symptoms clearly demonstrate the presence of a disequilibrium, or *imbalance*, in the nutrition.

In the past great stress has been laid upon Liebig's *Law of the Minimum*, which states that the nutrient in the relative minimum determined the rate of growth. This is a very useful law, which is completely valid, and of great use in science. However, there is a danger that it does not go far enough, because it does not stress the influence that excess has on phenomena such as growth. This omission is made good by Voisin's [315] *Law of the Maximum*, which states that 'the nutrient present in the relative maximum also determines the yield'. This means that 'excess of certain nutrient elements limit the yield, but this can be combated by supplying fertilizers which contain elements which neutralise the effect of the element in excess'. It was necessary to formulate this *law of the maximum*, because yield diminutions and induced deficiencies are fairly widespread today. Very often they are the result of injudicious fertilizer practices by persons not aware that over-fertilization may be detrimental.

When all these factors are taken into account, when it is remembered that antagonisms between the elements exist, and that *balanced* nutrition can occur at various levels of nutrition, then it must be clear that it is quite impossible to determine *absolute* levels for either deficiencies or excesses in any given species. This does not mean that for certain known conditions it is impossible to assess upper and lower limits for such elements. This is frequently done with very rewarding results. But the fact that *the balance of nutrients* is the important factor in normal development precludes absolute levels being established.

IV | Yields in relation to trace elements

Essential nutrients and yields

The fact that the yields of crops, and the size and vigour of animals, are dependent on their nutrition is well known, and is the basis of the practice of fertilizing agricultural land and of supplying supplementary feeding to animals. The aim of these practises is to supply any of the missing factors that are limiting optimal development, and the productivity of good modern agricultural techniques demonstrates the efficiency of these procedures.

As trace elements are *essential* nutrients, and hence their relative deficiency or excess can potentially influence optimal development to a considerable extent. That this is actually the case, and that trace elements are in fact the *limiting factor* in determining yield size in many places is quite clear, as the following paragraphs will show.

There are always trace elements present in the soil, but the supplies of these may be inadequate for agriculture: there may be an imbalance of nutrients; the essential elements may not be *available* for a number of reasons. Whatever the cause may be, disturbances in trace-element nutrition result in great economic loss in many parts of the world by preventing proper stock and crop development.

Liebig's Law of the Minimum makes it clear that the rate of growth and development is controlled by the nutrient in *relative* minimum, while Voisin stresses the growth inhibition caused by *excess*. Thus, imbalances of nutrients will result in growth and development being far short of optimal.

In most agriculture in the temperate parts of the world the major nutrients, particularly nitrogen and phosphorus, constitute the major nutritional problem. They are widely deficient, and their fertilizers are extensively used in modern agriculture. Their importance in agriculture is generally appreciated, even by the urbanized layman with no practical knowledge of farming. Any simple book on the subject, such as Sir E. J. Russell's small but very readable *The World of the Soil* [250], clearly indicates the great importance of the major nutrient elements. They are not discussed here because they are outside the scope of this work, but this does *not* imply that they are not important. Trace elements are not a novel cure-for-all, and they must be seen as part of the general problem of mineral nutrition. *All*

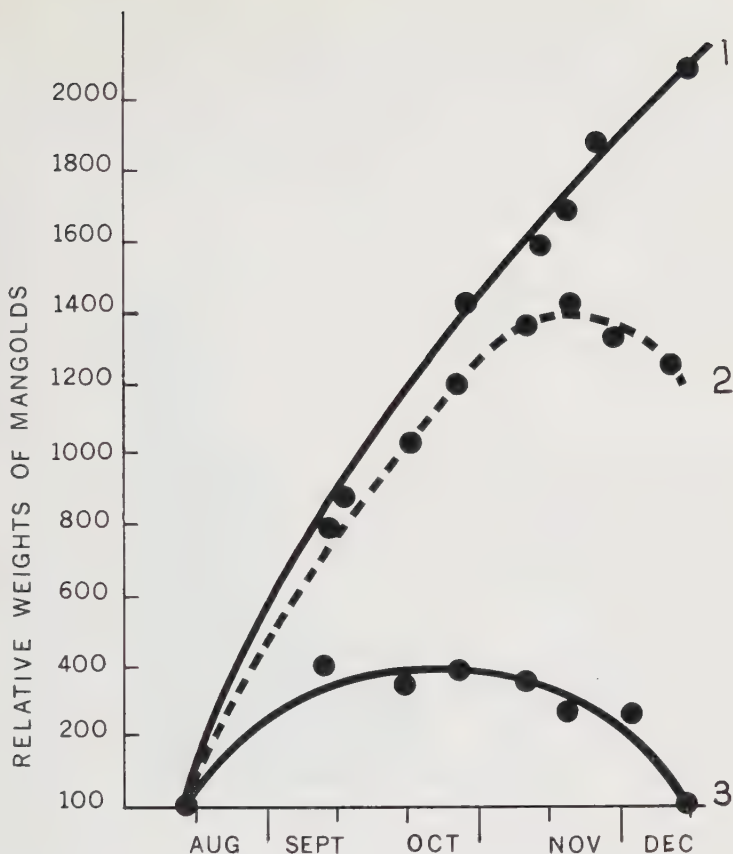


FIG. 12

SCHÜTTE [262]

The influence of boron on the weight of mangolds

Brandenburg's original experiments investigating the influence of boron on the yield of mangolds showed that plants supplied with boron grew well until harvested, while those lacking it showed much poorer development.

1. Watered with nutrient solution containing 1 mg. H_3BO_3 per litre for entire experiment. No deficiency symptoms were observed.

2. Nutrient solution deficient in boron from October to December. Deficiency symptoms observed in November, after the plants had started to lose weight.

3. Nutrient solution lacking in boron for entire experiment. Deficiency symptoms set in at the beginning of October. It is quite clear that crop production is seriously retarded before visual deficiency symptoms set in.

essential nutrients can influence yields, but only the *minor* elements are discussed here.

Increase of plant yields by trace-element applications

Experimentally, it can be demonstrated very clearly that trace elements can limit yields. A typical example is given in Fig. 12, showing the influence of boron upon mangold yield. Plants grown in

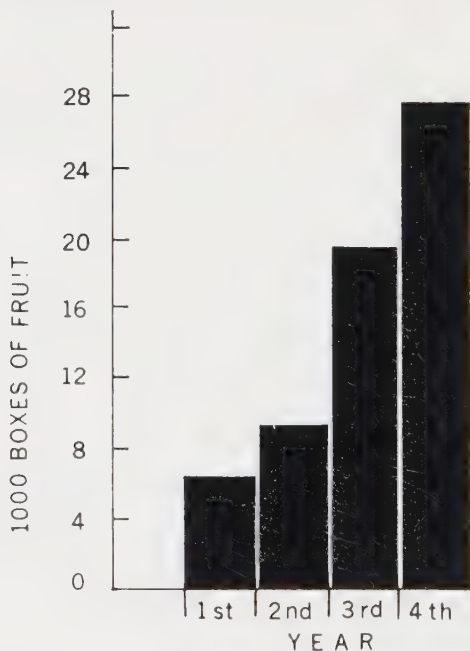


FIG. 13

SCHÜTTE [262]

The influence of zinc and manganese sprays on the yields of apples and pears

A fruit farm in the Cape Province, South Africa, with adequate soil applications of N.P.K., became 'worn out'. Yields remained consistently low. Sprays of zinc and manganese compounds caused marked increases in the yield of apples and pears. It is worth noting that it can take more than one season to obtain the full beneficial results of spraying.

solutions lacking in boron grow very slowly, and as they use up their reserves, lose weight and die. Adequate supplies of boron give good, rapid growth, and hence good yields.

The histogram in Fig. 13 shows very clearly the beneficial consequences of spraying very dilute solutions of zinc and manganese on fruit trees suffering from acute deficiency of these elements. After

years of normal fertilization with nitrogen and phosphorus fertilizers this apple and pear orchard was producing steadily decreasing crops. Simply by curing these deficiencies the fruit production rose from 6,000 to 26,000 boxes within four years. This 450 per cent increase in production was achieved on a farm, and not in a laboratory or research station. It shows very clearly the detrimental effects that trace-element deficiencies can have on fruit production and how striking the crop increases are when they are cured. As can be seen, the beneficial consequences of the treatment take several years to be expressed fully in fruit trees. The economic benefits to the farmer are obvious, as these improvements are the result of three foliar sprays of zinc and manganese salts per year, a procedure not involving much extra expenditure, as they can be mixed with other sprays normally used in this type of farming.

Other crop yield data

In case this example may be considered unique, it is only necessary to refer to some of the many other cases cited in the literature. Gonzales [115] reports that curing the boron deficiency in coffee on the central plateau of Costa-Rica gave crop increases of 285 per cent. In Australia, where such widespread trace-element deficiencies occur, Anderson [5] reports the beneficial consequences of curing molybdenum deficiency in pasture clovers. In Australia molybdenum deficiency is widely linked with phosphorus and sulphur deficiency. *Until these deficiencies were cured by application of fertilizers to the soil the addition of molybdenum to the crop had hardly any effect.* It cannot promote growth if other mineral nutrients are deficient and limiting growth; but when the major nutrients were supplied in adequate quantity, then the application of 2 oz. of molybdenum per acre increased the yield of annual subterranean clover from 829 to 1,523 lb./acre of dry matter (930–1,687 kg./ha.). In another experiment the increase was from 1,800 to 3,697 lb./acre (2,128 to 4,144 kg./ha.). These spectacular increases were due to such a small quantity of molybdenum that a real practical problem was the distribution of such small quantities. Table 9 shows the benefits derived from the use of molybdenum on certain New Zealand soils.

Overall increases in yield are not always a good criterion for measuring beneficial results. Copper-deficiency results in stunted cereals, but too large a dressing of copper may result in decreased yields. Weight increases may be achieved by greater straw production and diminished grain formation [248]. Fig. 14 depicts the growth response to copper in oats. This variety responds well to copper and is tolerant of fairly high levels of this element, although this depresses the yield. It shows clearly the influence of too little and of too much copper. Roach [238] describes the largest crop of mangolds that he

ever saw (over 60 tons/acre) as the most severely manganese deficient he had experienced in the field. Curing the deficiency did *not* increase the yield. Trace-element balance does alter the ratio of tops to shoots, and if no marked diminution of quality were involved might be a way of promoting desirable results in root crops. But in practice, such

TABLE 9

Potential crop improvements in Waitaki County, New Zealand, by the use of molybdenum fertilisers. From LOBB [170]

CROP AND SOIL	YIELDS		% INCREASE
	CONTROL, NO MOLYBDENUM	WITH MOLYBDENUM	
Lucerne on sandstone soil	3,060	13,920	355
Lucerne on clay soil	13,664	19,488	43
Rape on sandstone soil	7,616	53,536	603
Rape on clay soil	8,288	14,784	78
Pasture on clay soil:			
a	3,300	6,233	89
b	2,038	9,357	359
c	3,561	17,450	390
d	995	1,505	51
e	4,968	7,028	44
f	2,080	2,868	38
g	13,700	20,500	50
h	12,200	18,800	54

Crop yields are given in lb./acre green matter. Dosage of molybdenum used not given.

All the soils and crops treated gave a marked response to molybdenum. Both clay and sandstone soils gave crop increases of over 300 per cent.

crops frequently result in manganese deficiency of animals feeding on them. However, in many parts of Africa and America beneficial yield increases have been obtained with the known essential trace elements, boron, copper, manganese, molybdenum, and zinc in a very wide variety of crops.

The point may be made that in well-developed agricultural areas, such as Europe and parts of the U.S.A., such spectacular results are not likely to be found. This contention is not true, as many highly developed zones are starting to suffer from induced deficiencies due to current agricultural practices. These will be discussed later in Chapter XII. It is true that in Europe on agricultural land acute deficiencies are not as widespread as elsewhere. Yet even in Holland, which is generally recognized as the most progressive agricultural country in Europe, Lehr [168] claims that 50 per cent of the land is potentially deficient in trace elements.

Brandenburg [46] obtained over 90 per cent increase in sugar beet

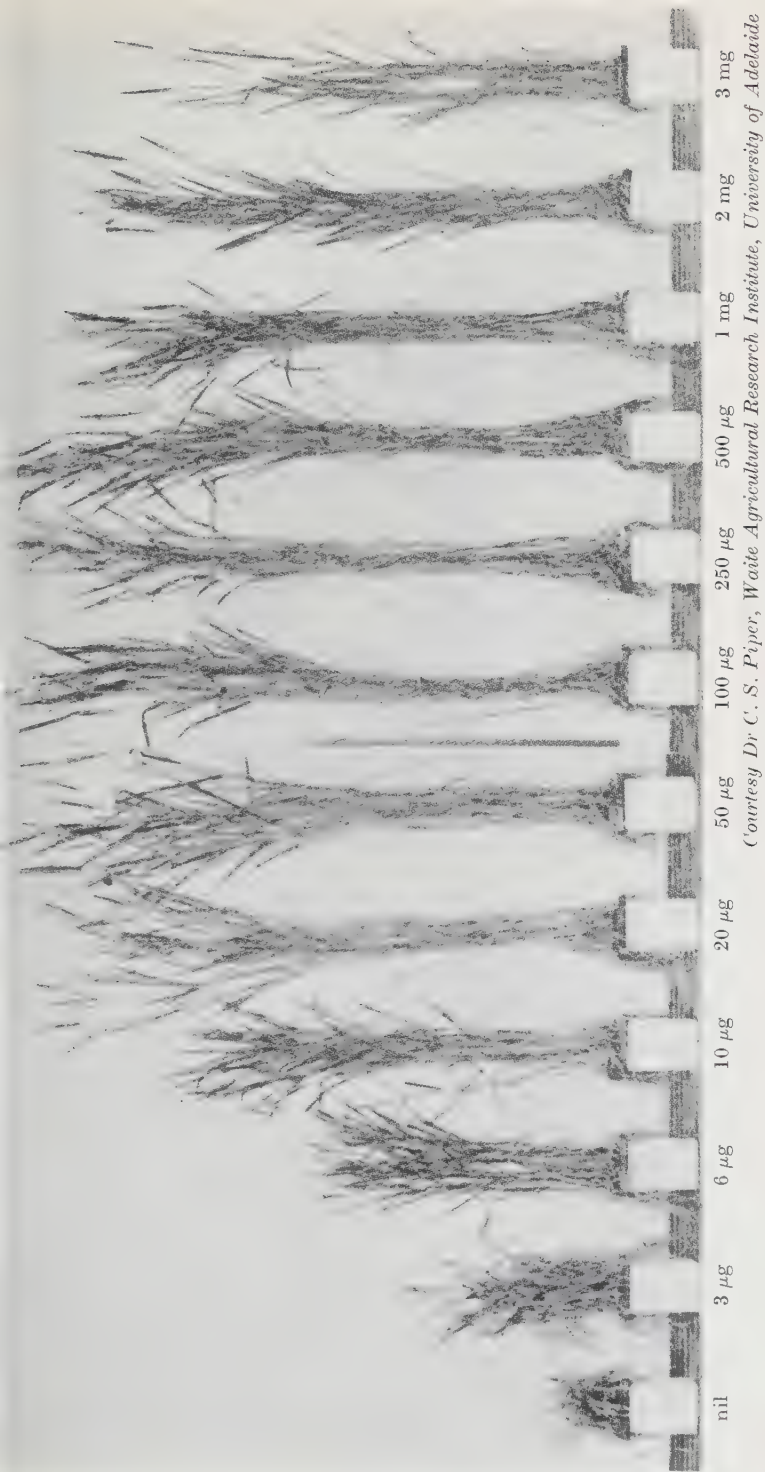


FIG. 14

The effect of deficiency and excess of copper on the growth of oats

This picture shows the influence of copper on the growth of oats. With increasing copper concentration there is increased vigour and size until an optimum is reached at about 100 m.g. copper in solution. Increasing concentrations much beyond this decreases the yield appreciably, as can be clearly seen in the right-hand samples. The oats in this experiment show a marked tolerance to high levels of copper, as even at 3 mg. copper growth is still reasonable, even if not optimal.

crops on deficient soils in Germany by boron application to the soil. As the application of 18 lb./acre (20 kg./ha.) of borax not only gave these increases but also increased the sugar content of the beets, the economic gain was a very appreciable one. In Britain during the war years Roach [238] obtained large crop responses from manganese

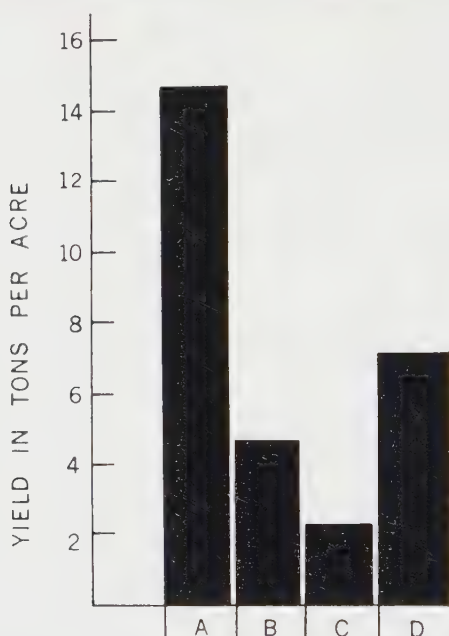


FIG. 15

SCHÜTTE [262]

The influence of boron in various fertilizer mixtures on the yield of sugar beet

This histogram shows the influence of micro-nutrient fertilization upon crop yields, where deficiencies exist and the response to various fertilizer treatments obtained with sugar beet, in an experiment in Eire.

- A Farmyard manure + artificial fertilizers + lime + 20 lb. borax per acre—14 tons 12 cwt. beet per acre.
- B Farmyard manure + artificial fertilizers + lime—4 tons 12 cwt. per acre.
- C Farmyard manure—2 tons 8 cwt. per acre.
- D Farmyard manure + artificial fertilizers—6 tons 15 cwt. per acre.

applications to potatoes, wheat, oats, and peas on newly ploughed pastures. Russell [250] cites how cereal production rose from 2-3 cwt. to 30 cwt./acre on copper-deficient peat fen soils in the Eastern Counties of England, when 1 lb./acre (0.9 kg./ha.) of copper sulphate was applied. In Eire the beneficial response to boron in sugar beet

is clearly shown in Fig. 15. It also shows the depressing effect that liming can have on yield, unless precautions are taken to prevent it inducing a deficiency.

Latent trace-element deficiencies

The widespread distribution of trace-element deficiencies is not generally recognized, even by many investigators, because too much stress has been laid on visual clinical symptoms, such as chlorosis of the leaves or abnormal growth. These symptoms are of the greatest

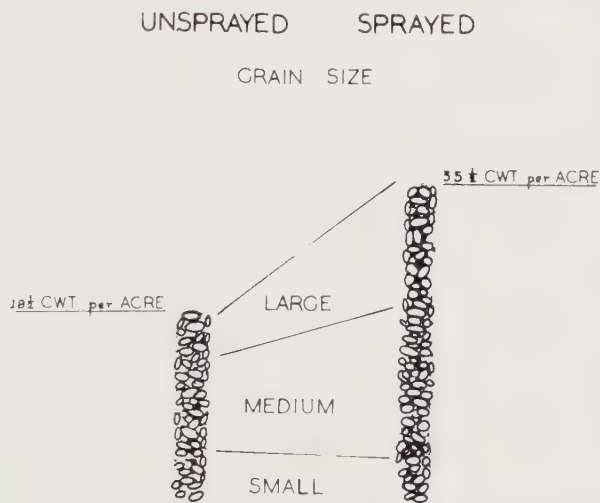


FIG. 16 ROACH [240]

Effect of spraying with manganese sulphate solution on yield and grain size of wheat

The influence of manganese fertilization on the yield and the quality of wheat grown on a manganese-deficient area. The percentage of medium and large grains has increased as a result of spray treatment, while the percentage of small poorly developed grains decreases. The unsprayed plants showed no visible deficiency symptoms.

value in indicating the presence of nutritional deficiencies, *but it must be appreciated that not all deficient plants show them*. Deficiencies not acute enough to show visual signs may be severe enough to halve the potential yield. Nieschlag [213] found that application of manganese sulphate to *apparently healthy* potatoes doubled his crop. Roach [240] stresses the fact that the yield responses which are depicted in Fig. 16 were obtained by the application of manganese to wheat that showed no signs of deficiency. Yet yields of grain were doubled. This type of effect has been observed by many workers, not only for plants but also for animals.

These *latent* i.e. non-visible, deficiencies exist and are amenable to treatment, and their cure results in very appreciable yield gains. These latent deficiencies are not easily detected, and so remain quite unsuspected as the cause of low fertility on many farms. However, most areas bearing visually deficient plants or animals are surrounded by areas in which the vegetation or the animal life is apparently healthy, but where yields are low. These are regions of latent de-

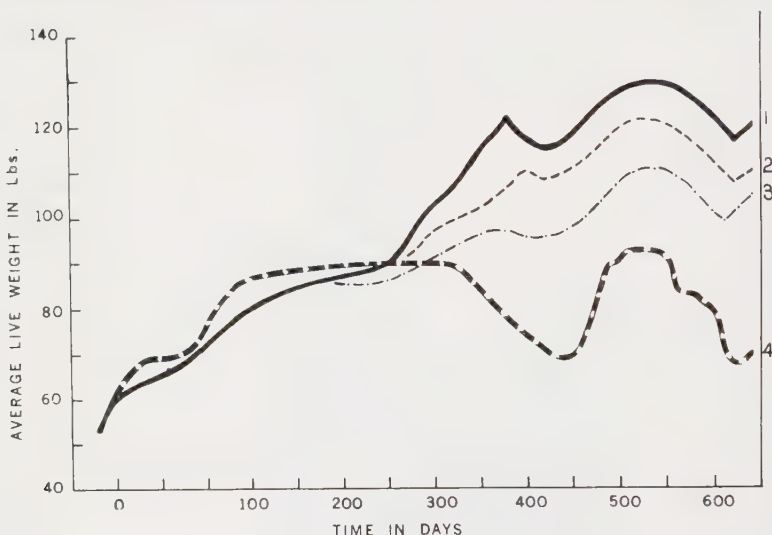


FIG. 17

AFTER HOPKIRK AND PATTERSON [137]

The influence of cobalt on the growth of sheep

- | | |
|--|--|
| 1. Cobaltized superphosphate. | 3. 4 oz. $\text{CoSO}_4 \cdot 7 \text{H}_2\text{O}$ /acre. |
| 2. 8 oz. $\text{CoSO}_4 \cdot 7 \text{H}_2\text{O}$ /acre. | 4. Control: no cobalt. |

The body weight of sheep can also be limited by lack of trace elements. In this graph the weight increases of animals grazed upon pastures receiving various levels of cobalt, an essential element for sheep but not for plants, is recorded. The Sherry River Valley, where these experiments were undertaken, is a cobalt-deficient area, as the control experiment shows. Under conditions of much severer deficiency much more dramatic weight difference can be demonstrated.

iciency, and are the cause of great economic losses because they are producing well below their potential crop output. If the condition is recognized marked improvements can be introduced. As these zones of latent deficiencies are extensive, and potential crop yields can be halved before deficiency symptoms appear to warn the farmer, the importance of recognizing these areas for what they are is obvious.

This problem of latent deficiencies is more complex than the simple description given would indicate. The antagonistic effects which

nutrients exert against each other (cf. page 41) and the consequences of certain fertilizer practices (cf. Chapter XII) all tend to complicate the simple picture. But the fact that latent deficiencies go undetected is only too clear. One of the causes being that comprehensive fertilizer trials involving all the known nutrients are seldom undertaken, and then often on sites that are not always well chosen.

Animal growth and trace elements

What has been written about plants applies equally to animals, although less accurate information is available. The diet of animals is

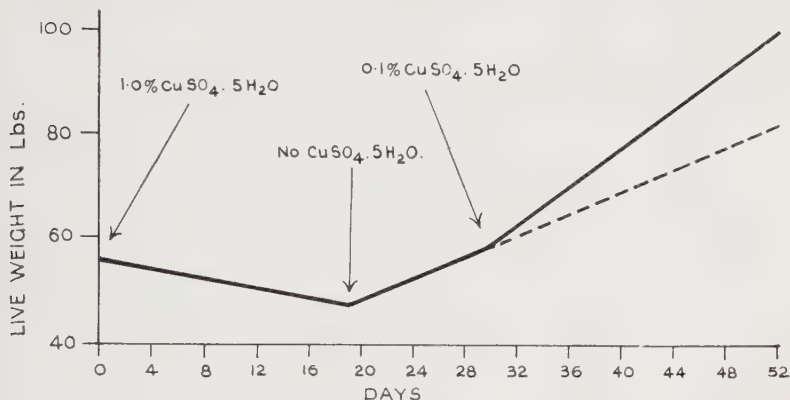


FIG. 18

FROM BARBER *et al.* [27]

Inhibition and stimulation of growth in pigs by the supplementation of their diet with different levels of copper sulphate

High levels of copper sulphate, i.e. 1.0 per cent, in the diet of pigs inhibit their growth. When these animals were fed no copper their growth rate returned to normal. But when fed copper sulphate at levels of 0.1 per cent they showed appreciable growth responses.

more complex than that of plants, as they do not photosynthesize and produce their own organic matter as plants do. They are thus dependent upon outside sources not only for their minerals but also for organic nutrients, such as proteins, fats, carbohydrates, and vitamins. These are all essential constituents of their diet, and it is not easy to study trace element in such a complex of essential nutrients. Furthermore, certain animals, such as ruminants, have very marked requirements as to the form in which they receive their nutrition, and to date no completely synthetic diet has been devised for them, making many types of investigation quite impossible.

As far as body weight is concerned, the picture in animals replicates the position found in plants. The graph in Fig. 17 depicts the influence of cobalt upon the live weight of sheep. This element, which is

not essential for plants as far as we know, is very important in animals, particularly ruminants. It is required in very small quantities. The deficient sheep were very unthrifty, but the application of

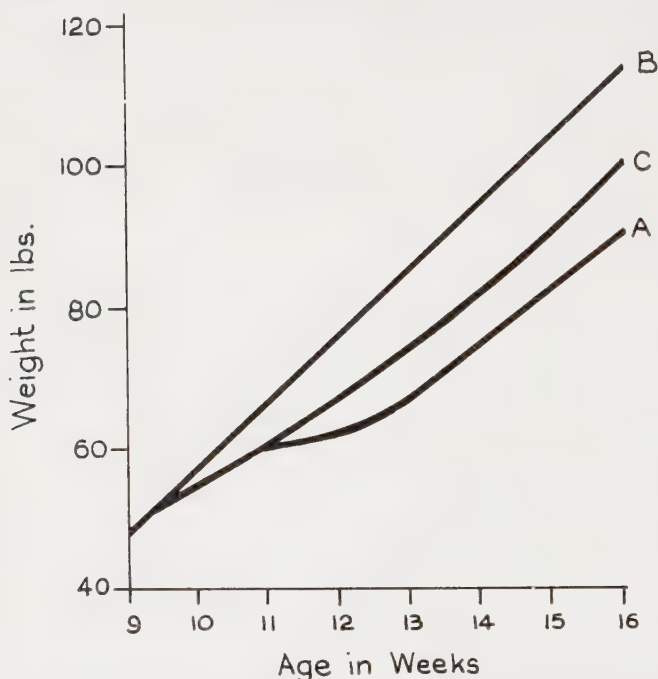


FIG. 19

BELLIS AND PHILP [30]

The influence of zinc supplementation in the diet on growth of young pigs

Zinc promotes growth in young growing pigs. This is clearly shown in this graph where three groups of nine-week-old piglets were studied. They were treated as follows:

- A—unsupplemented diet.
- B—zinc supplementation from 9 weeks.
- C—zinc supplementation from 12 weeks.

There were forty pigs in each group, and the supplementation consisted of 0.02 per cent ZnCO_3 (100 p.p.m.) in the diet. Within a week of receiving the zinc (in Group C) the piglets showed a marked response in growth rate, and the deficiency symptoms, such as scab, started to diminish.

cobalt to the soil, and hence to the herbage, resulted in almost a doubling of the body weight of full-grown sheep [137].

Copper is an element that plays an important part in pig farming. It is often included in feeds or concentrates. Fig. 18 depicts the influence that copper has on growth. Too high a level definitely

inhibits growth, while low concentrations stimulate it [27]. Zinc deficiency can also occur in pigs, and in Fig. 19 the influence of zinc upon the growth of young pigs is graphically depicted. This deficiency was severe enough to cause clinical symptoms such as scab in both groups A and C. These investigations [30] also showed that a high calcium content in the diet increased the requirement for zinc. Calcium *antagonizes* zinc in the diet.

There is still some doubt as to whether selenium is absolutely essential for mammalian development, although it seems to be very probable. It certainly has a pronounced effect upon growth and health, especially in certain types of unthriftiness in cattle and sheep.

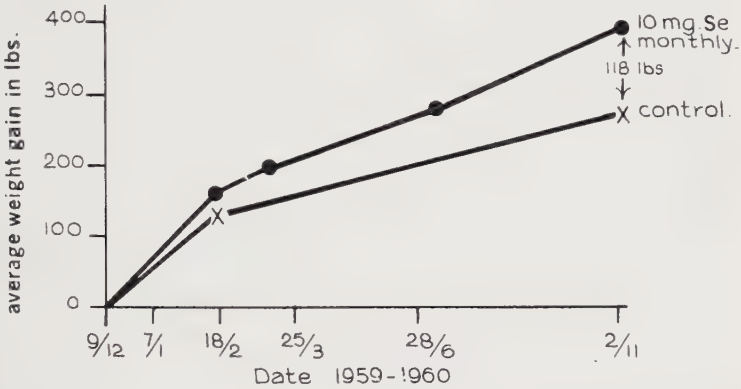


FIG. 20 HARTLEY [124]

Effect of oral selenium on the growth rate in Aberdeen Angus calves

This experiment shows the beneficial effect of 10 mg. selenium per month upon the growth rate of calves.

Further experiments indicate that subcutaneous injections of 20 mg. selenium every four months may be sufficient to give the same effect.

The effect of oral selenium supplementation is strikingly obvious in the Aberdeen-Angus calves studied by Hartley [124]. In less than a year the animals receiving selenium weighed over a third more than the controls not supplied with this element (Fig. 20).

Induced trace-element deficiency in animals

The fact that high levels of molybdenum can induce a copper deficiency in ruminants was mentioned in Chapter III, page 36, where the copper deficiency on teart lands in England was mentioned. A similar condition in Nevada, U.S.A., was termed ‘molybdenosis’ or molybdenum toxicity. This toxicity induced a copper deficiency, as molybdenum *antagonizes* the copper, and so in practice, copper

injections to raise the copper level of the stock to an adequate level do in fact combat the high level of the molybdenum in the herbage, and enable the animals to feed on it and thrive. The graph in Fig. 21 shows the growth of two calves fed on this molybdenum-rich fodder. The animal given copper glycinate injections grew at a very good rate, while the calf lacking the injection lost weight.

Copper, molybdenum, and sulphur have a complex interaction in the diet. Sulphate enhances the antagonistic effect that molybdenum has for copper in ruminants. In rats sulphide increases the antago-

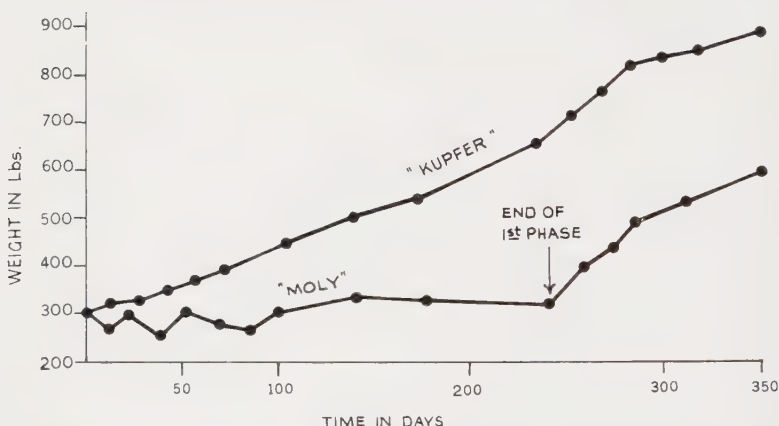


FIG. 21

DYE AND O'HARA [94]

Combating high levels of molybdenum in pastures by copper treatment of the grazing animals

This graph shows the growth curve of a heifer 'Kupfer' which received injections of copper glycinate, compared with that of 'Moly', a similar crossbred Angus Hereford heifer which did not receive these injections. Both were fed on the same molybdenum-rich fodder. Both had an initial weight of 295 lb., but 'Kupfer' gained on an average 1.74 lb./day, while 'Moly' gained only 0.7 lb./day.

In the Second Phase 'Moly' was given similar copper treatment and gained weight rapidly.

nistic effect of molybdenum. In Fig. 22 the effect of high levels of molybdenum on the growth of rats is shown. The inhibitory effect is increased by even a low level of sulphide. And the retarding effect of both can be overcome by increasing the level of copper present [192]. Obviously the copper requirement of an animal cannot be assessed solely by determining its copper content. Antagonistic interactions of this kind are found in animals just as they are in plants, cf. Fig. 10. Mulder's Interaction Chart.

Grashuis [117], in a very interesting article on the agricultural industry in post-war Holland, discusses the consequences of fertilizer-

induced deficiencies, which were then a serious problem. *Over-application* of fertilizers caused the balance of soil nutrients to be seriously disturbed and resulted in the growth of vegetation that was detrimental to the well-being of cattle. The milk yields dropped and the animals developed deficiency symptoms of various kinds. Egg production in poultry was similarly affected. Of great importance was the fact that due to a manganese deficiency the reproductive cycle of cows was seriously disturbed. In an investigation covering 450

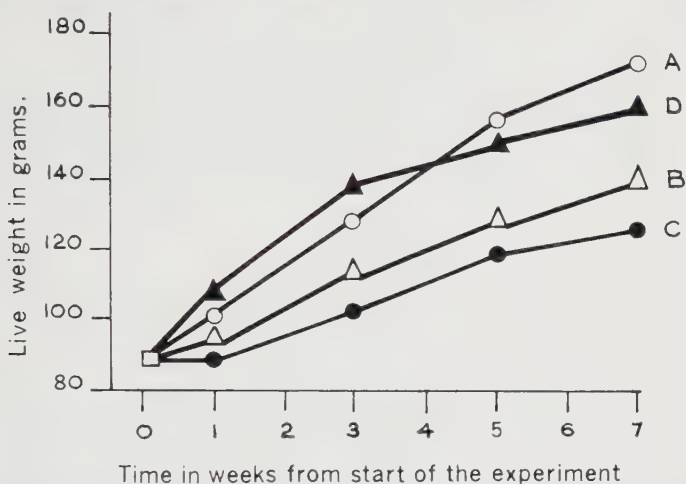


FIG. 22

MILLS [192]

The influence of various combinations of copper, molybdenum, and sulphide on the growth of rats

A = 3 p.p.m. Cu + 2 moles. sulphide/gr. diet.

B = 3 p.p.m. Cu + 35 p.p.m. Mo/gr. diet.

C = 3 p.p.m. Cu + 35 p.p.m. Mo + 2 moles. sulphide/gr. diet.

D = 25 p.p.m. Cu + 35 p.p.m. Mo + 2 moles. sulphide/gr. diet.

(A) depicts a normal growth response in rats. The addition of high levels of molybdenum to the diet results in a marked decrease in growth (B), and the inclusion of a small amount of sulphide (C) enhances this molybdenum inhibition very markedly. However, the addition of higher levels of copper to the diet antagonizes this growth inhibition and gives rise to almost normal development (D).

farms 20 per cent of the cows had a conception rate of less than 50 per cent after the first insemination. As natural propagation is an expression of yield in farm animals, this reflects a serious decrease due to an induced trace-element deficiency.

Induced trace-element deficiencies in plants

Plants show the same type of induced deficiencies as animals do. In fact, induced deficiencies are so widespread that they constitute a

serious problem, and will be discussed in more detail in Chapter 12. Attention has already been drawn to the antagonistic effect of certain of these elements (Fig. 10).

Overliming is the commonest cause of induced trace-element deficiency. The drop in pH caused by this practice results in all the trace elements except molybdenum being made less available to plants. Excess potash fertilization results in an induced boron deficiency. As a result, crops receiving heavy potash fertilization have a higher boron requirement, and unless this can be met from the soil deficiencies must result. In some of the states of the U.S.A. boron is added to the potash fertilizer to meet this extra need.

The photographs in Fig. 23 show the consequences of high levels of potassium in the presence or absence of boron upon soyabean growth. The marked *depression* in growth resulting from excess potassium is very obvious, yet growth at these levels is excellent if a sufficient supply of boron is available. This is a very clear example to show that the boron requirement of these plants is not absolute, but is linked to the level of other nutrients, such as potassium in this case.

Phosphorus-induced deficiency is well known, and the data in Table 10 show clearly the consequences of upsetting the nutrient balance.

TABLE 10

Leaf composition and shoot growth of sour-orange seedlings grown in soils treated with different levels of phosphate. From BINGHAM et al. [40]

PHOSPHATE APPLIED PER ACRE,		SHOOT WEIGHT, G.	LEAF COMPOSITION				
LB.	pH		B, P. P. M.	Cu, P. P. M.	Fe, P. P. M.	Mn, P. P. M.	Zn, P. P. M.
0	5.7	31	35	5.3	54	32	28
76	5.6	34	42	4.5	52	28	30
360	5.6	26	30	2.0	72	37	20
900	5.4	22	28	1.0	51	44	21
1,800	5.2	14	23	1.4	72	77	12

The addition of phosphates lowered the pH of the soil slightly, yet copper, boron, and zinc uptake were depressed. Manganese uptake was stimulated, and iron was not seriously influenced.

Heavy dressings of phosphates can upset the nutrient balance of plants very severely and even induce deficiencies.

Heavy applications of phosphates can induce marked deficiencies, especially of zinc and copper. Again it must be mentioned that if notice is taken of this antagonism its detrimental effects can be combated.

Some other yield responses

Milk yields and egg production are other examples of yields that interest farmers. These are also influenced by such minor elements as



Picture, courtesy Better Crops with Plant Food, Magazine of the
American Potash Institute

FIG. 23

WOODRUFF *et al.* [333]

The influence of varying levels of potassium on the growth of soyabeans, in the presence and absence of added boron

Picture (*top*) clearly shows the inhibiting effect that high levels of potassium have on soyabean development. When the soil has 2 per cent saturation inhibition sets in, because a relative boron deficiency is induced.

If the boron level is increased, then the addition of potassium does not inhibit growth, as the plant has enough boron to meet its requirements (*lower picture*), even at high levels of potassium. It is worth noting that all the plants that have received boron supplements, even those below 2 per cent potash saturation, have a much lusher appearance.

copper, manganese, and iodine. The dietary requirements of dairy cows depend on their milk yield, and is much greater than that of other cattle. Dairy cows in milk require from 2 to 20 mg. iodine per day, depending on the milk production, while other cattle only need 1 to 5 mg. iodine daily [15]. Lack of this decreases milk yield.

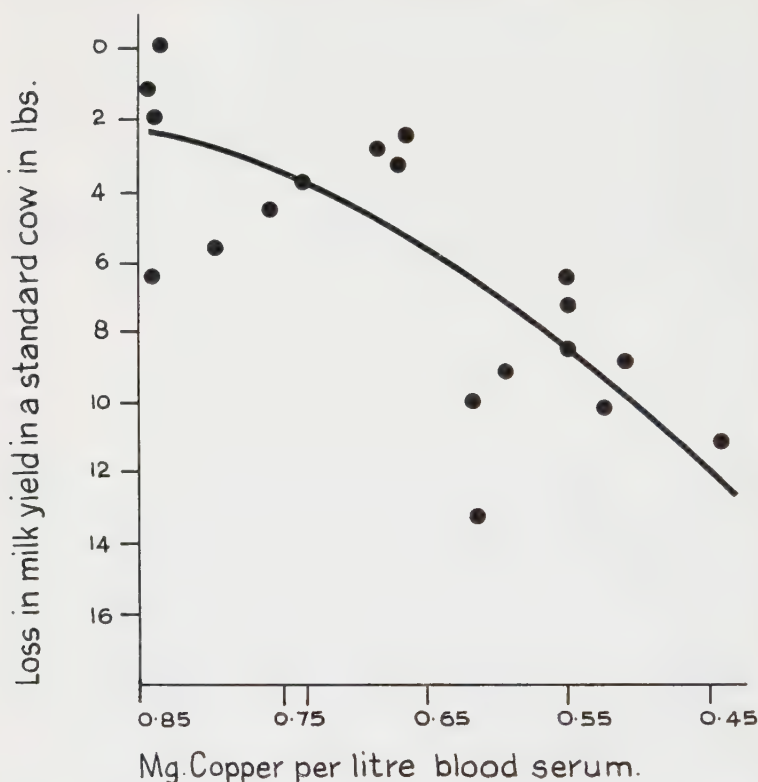


FIG. 24

AFTER DELJS [84]

The interrelationship between milk yield and blood serum copper

This graph shows clearly that the average milk yield is seriously lowered as the copper content of the blood serum decreases. Adequate supplies of copper are obviously required to maintain optimal milk production.

Milk yield is also seriously influenced by the copper content of the vegetation upon which the cow grazes. If the lactating animal suffers from even a mild latent deficiency of this element the milk yields drop appreciably. Fig. 24 shows the fact that the milk yield is related to the blood-serum copper content, and that the lower the serum copper, the lower the yield [84].

In poultry manganese nutrition is a problem in some parts of the world. Its deficiency leads to lowered egg production, lowered hatchability, and also lowered eggshell strength. The fact that high levels of calcium and of phosphorus, which are present in certain feed concentrates, induce a manganese deficiency can lead to severe economic losses [16].

Not that yields are the only criterion of success in farming. It is the cash value of the crop that is important. Large floppy plants or animals are often disease prone and not desired on the market. Un-sightly or unpalatable materials, even if produced in large quantities, are seldom desired by the producer or buyer. Besides yield, the quality of the product must be considered if we are to decide what is desirable.

It has been shown that essential trace elements do have a very pronounced influence upon yields of both animals and plants. Chapter V will show that they play an equally important part in determining the *quality* of animal and plant materials.

V | Quality of plant and animal products in relation to trace-element nutrition

In Chapter IV the very marked influence that trace elements can have upon growth and yield under certain circumstances was clearly shown. Another aspect of these micro nutrient elements is the very considerable effect that they can exert upon quality. They can alter quality to such an extent that the goods produced are hardly marketable, or they can influence the quality more subtly by varying such unseen nutritional factors as vitamin content and altering the biological quality.

Unfortunately quality is a difficult concept to define accurately, and it is seldom used in scientific work. It is a characteristic that can be expressed in a multitude of ways. The dictionary definition of quality as 'a degree of excellence' is not very helpful when applied to biological matter. But in spite of this, it is possible to discuss some cases in which quality is obviously involved.

Taste

In foodstuffs any taste that is not acceptable is soon detected. No matter how pleasant a fruit or vegetable may appear, if its taste is not what it should be it is of poor quality. Manganese deficiency gives a very unpleasant taste to potatoes. Boron deficiency in turnips gives a bad taste to the roots, which also suffer from the disadvantage that they are glassy in appearance and do not soften on boiling [248 and 319]. Deficiencies of this element also result in 'corky pit' disease of apples, in which the skin is roughened and gritty particles are included in the flesh, thus ruining the quality of the apples. Severe boron deficiency results in wrinkled, cracked fruit as shown in Fig. 25, where 'drought spot' has resulted. In oranges lack of boron results in a thickened rind with discoloured patches.

Not all vegetation that is obviously deficient has a bad taste. Manganese deficiency in peas and beans gives rise to 'marsh spot', a condition in which the inside of the seeds develop a characteristic brown colour, yet which hardly impairs the flavour. Yet slight alterations in taste with deficiencies are quite common.

This alteration of taste is not confined to vegetable products. The taste of dairy produce is often altered by small quantities of copper,



Photos by courtesy of Borax Consolidated Ltd

FIG. 25

Bad appearance of boron-deficient apples

'Drought Spot' is a condition brought about by boron deficiency in apples. The contrast between the wrinkled, cracked fruits and those of the healthy fruit on the left is too great to need any stressing. The economic loss due to this condition is obvious.

but they are usually present as contaminants introduced by machinery. More than 1.5 p.p.m. copper in milk rapidly gives rise to a rancid tallowy taste, although lower concentrations of 0.2-0.5 p.p.m. are compatible with good keeping quality [306]. Copper levels of over 2.0 p.p.m. give cheddar cheese a very metallic flavour [286].

Biological quality in foods

A very important aspect of crops is their food value, and this is not readily determined by simply inspecting and tasting materials. The mineral and vitamin content, the amount and nature of the carbohydrates, fats, and proteins all contribute to the nutritional value of crops as foods, but the determination of these must be undertaken in the laboratory.

Mineral content

As has been mentioned, the mineral content of plants and animals varies considerably. There are marked differences between species based upon genetically determined differences in requirements. Even within a species, the genetically determined differences can be very

large, as Table 11 shows. The sixteen varieties of wheat tested showed a range of copper contents from 5.6 to 16.7 p.p.m. copper [118],

TABLE 11

Variation in the copper content of different varieties of wheat grain grown on the same soil. From GREAVES AND ANDERSON [118]

VARIETY OF WHEAT	P.P.M. COPPER IN GRAIN
Montana 36	16.7
Kubanka	16.4
Turkey 926	13.9
Tenmarq	12.3
Kharkov Hayes 2	11.8
Newturk	11.0
Regal	9.4
Early Baart	9.3
Karamont	7.8
Black Hull	7.6
Hard Federation	7.4
Marquis	7.0
Kofod	6.5
Alton	6.4
Chul	5.9
Kofod $\times$ Turkey	5.6

While other factors, including soil composition, can alter the copper content of the grains, one among the main factors in governing the copper content is genetical. There are marked differences between varieties.

although they were grown on the same soil and under the same conditions.

The genetical composition of plants only determines the mineral content within very wide limits, and within these limits great differences are possible. Soil and climatic differences also play a very important part, and in Table 12 the effect of different sites, all in Hertfordshire in England, show clearly that even in such a small area marked differences in mineral composition are to be expected. The manganese content of these wheats ranges from 1.51 to 5.83 mg./100 g. for instance, in this area.

It is not surprising that if the mineral content of farm produce arriving in a large market like that of Washington, D.C., U.S.A., is investigated, even the *mean values* of the materials should vary as considerably as Hopkins and Eisen [136] found. These produce come from all over America, and variations due to soils, climates, and different varieties all contribute to the large range in mineral contents (Table 13).

TABLE 12

*Mineral constituents of wheat: effect of variety and site. From HORDER et al. [138]
(Mgm./100 gm.)*

VARIETY	SITE A		SITE B		SITE C		SITE D		SITE E		SITE F		AVERAGE PER VARIETY	
	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn
Bersée	3.96	5.54	4.25	1.51	2.98	1.87	2.94	3.98	3.08	2.83	3.72	2.42	3.49	3.03
Holdfast	3.94	5.83	4.26	2.43	3.52	2.65	3.38	4.40	2.82	3.06	3.48	2.84	3.57	3.37
Jubilegem	3.64	4.90	4.02	1.76	2.78	2.42	2.76	3.60	2.80	2.97	3.36	2.47	3.23	3.02
Juliana	3.88	4.70	4.10	1.70	3.40	1.99	2.78	3.72	2.64	2.69	3.26	2.41	3.38	2.87
Little Joss	4.90	5.16	4.77	2.09	3.86	2.46	3.04	3.91	3.36	2.89	3.76	2.65	3.95	3.19
Redman	3.98	4.98	4.44	1.57	3.36	2.11	2.84	3.54	3.02	2.56	3.62	2.60	3.54	2.89
Rivet	4.07	4.42	4.14	1.69	2.78	2.00	2.78	3.46	2.88	2.53	3.16	2.12	3.31	2.70
Squarehead II	4.10	4.53	4.42	1.91	2.90	2.27	2.92	3.90	3.52	3.11	3.40	1.91	3.54	2.94
Squarehead's Master	4.18	4.54	4.20	1.84	3.00	2.18	2.76	3.70	2.77	2.72	3.16	2.43	3.35	2.90
Average per site	4.41	4.96	4.28	1.83	3.18	2.22	2.91	3.80	3.01	2.82	3.44	2.43	—	—

Sites A-F represent different farms in Hertfordshire.

The figures show the appreciable differences in both iron and manganese content of various wheat varieties. The large variation in a small area like Hertfordshire makes it clear that large fluctuations in mineral content of plants due to variety, site, climate, etc., are to be expected and that averages mean very little unless comprehended in this context.

It is important to note that these variations exist, for these vegetables and fruits are also sources of essential minerals in the human diet.

TABLE 13

The mineral content in fresh vegetables sold on the Washington market in 1957. After HOPKINS AND EISEN [136]

	CARROTS	SWEET YELLOW CORN
Boron	0.13-0.17	0.04-0.10
Phosphorous	25-39	90-130
Magnesium	20-30	40-56
Manganese	0.10-0.52	0.15-0.25
Iron	0.27-0.60	0.34-0.72
Aluminium	0.04-0.15	0.05-0.31
Calcium	37-52	2.0-4.5
Copper	0.03-0.18	0.05-0.08
Sodium	18-72	0.14-0.24
Potassium	282-425	269-340

The produce came from varying producing areas at different times of the year. Element content expressed in mg./100 g. fresh weight.

This table shows the large variations that can exist in the mean value for any given element. These are due to the differences in soil type and climate of the regions in which they were produced. The table also shows the different composition of the two plants.

Vitamins

The fact that trace elements have a marked effect upon the vitamin content of vegetation is not as widely appreciated as it should be, since this is of considerable practical importance, as vitamin deficiencies are still widespread in both humans and animals.

While there are a number of references in the literature of the influence of unbalanced mineral nutrition upon the vitamin content of vegetation, there is not enough information available to enable a workable generalization to be made regarding the importance of trace elements upon the vitamin synthesis in field crops.

TABLE 14

The influence of copper upon the ascorbic acid and carotene content of green barley. After LUCAS [175]

	ASCORBIC ACID, MG./100 G.	CAROTENE, MG./100 G.
No treatment	29.2	4.00
10 lb. CuSO ₄ per acre	45.1	5.40
100 lb. CuSO ₄ per acre	46.5	6.60

Increasing copper applications result in appreciable increases of both ascorbic acid (vitamin C) and of carotene (pro-vitamin A) in barley.

Vitamin A and pro-vitamin A or carotene have been shown to fluctuate with various trace-element treatments. Lucas [175] obtained increasing carotene contents in crops such as spinach, wheat, barley, oats, and carrots by the application of copper, cf. Table 14. At the same time these increases could not invariably be obtained. Burger and Hauge [52] showed that increasing levels of manganese increased the carotene content of a number of plants, but only until any manganese deficiency present had been made good, and after

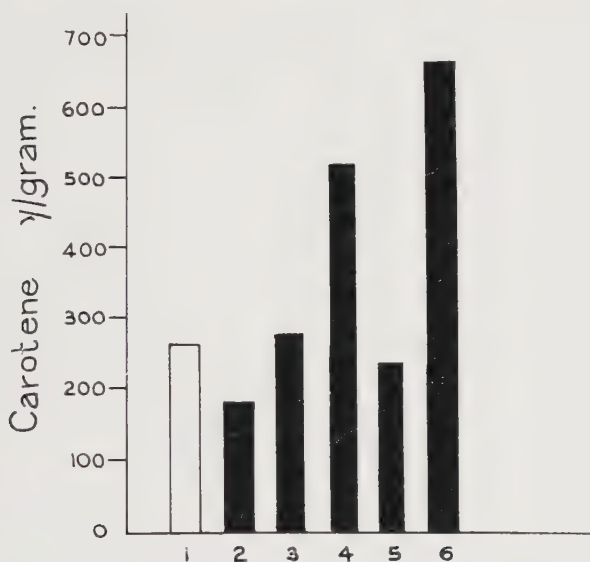


FIG. 26

AFTER BURGER AND HAUGE [52]

The influence of copper, manganese, zinc, and macro-nutrient fertilizers on the carotene content of soyabean leaves

1. No treatment.
2. N.P.K., 100 lb. of 3 : 18 : 9 per acre.
3. 100 lb. CuSO_4 + 40 lb. ZnSO_4 per acre.
4. 100 lb. CuSO_4 + 40 lb. ZnSO_4 + 212 lb. MnSO_4 per acre.
5. 100 lb. N.P.K. + 12 lb. CuSO_4 + 5 lb. ZnSO_4 per acre.
6. 100 lb. N.P.K. + 50 lb. MnSO_4 per acre.

The influence of soil applications of trace elements and macro-nutrient fertilizer upon the carotene (pro-Vitamin A) content is clearly shown.

N.P.K. are obviously not the *limiting factors*, as their application decreases the carotene content. Copper and zinc have only a slight influence on carotene concentration. Manganese with the trace elements copper and zinc, or with the macro-elements N.P.K., increases the carotene content considerably. In the same experiment manganese dressings lowered the choline (one of the Vitamin B complex) and increased the tocopherol (Vitamin E) level.

TABLE 15

The influence of various levels of manganese upon the vitamin C content of vegetables. From SCHARRER AND WERNER [253]

MANGANESE APPLICATIONS, PER POT IN MG.	GREEN CABBAGE MG. VITAMIN C IN 100 G. DRY MATTER	BEET	MUSTARD	BRUSSEL SPROUTS
0	670	329	893	480
10	730	359	1,077	556
50	720	397	694	517
100	716	321	779	497

Manganese applications resulted in an increase in vitamin C content in all the plants. However, all also clearly demonstrate the *inhibiting* influence of manganese once the optimum concentration has been exceeded.

that further additions had no effect on the carotene content. The effect of manganese is very striking, as can be clearly seen in Fig. 26, in its influence upon the carotene content of soyabean leaves.

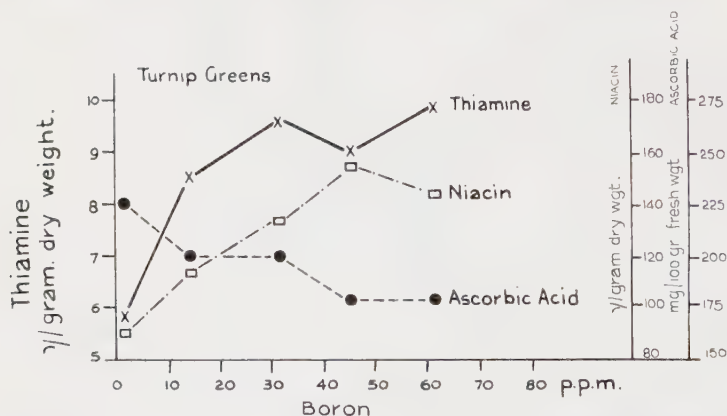


FIG. 27

AFTER LYON AND BEESON [178]

The influence of boron concentration on the vitamin content of turnip greens

The influence of boron on the vitamins thiamine, niacin, and ascorbic acid shown in above normal conditions, as from 15.5 p.p.m. boron upwards, toxic symptoms were observed. The main tendency is clear: thiamine and niacin increase with increasing boron concentrations, while ascorbic acid decreases. This is true below as well as above the toxic limit.

The vitamins of the B-complex are also influenced by trace elements. Boron levels influence the thiamine and niacin levels in turnip greens, as can be seen in Fig. 27. At toxic levels of boron the synthesis of both of these vitamins is stimulated, although vitamin C or ascorbic

acid production is decreased [178]. Manganese decreased the choline content of soyabean leaves, although it promotes Vitamin E (tocopherol) as well as carotene synthesis [52].

More is known about Vitamin C (ascorbic acid) than about the other vitamins. The addition of copper to deficient barley (cf. Table 14), oats, and *autumn-growth* spinach increases their Vitamin C content, but for some unknown reason does not do this in either tomatoes or *spring-grown* spinach [175]. Manganese appears to influence this vitamin very considerably in certain fruits, for von Bronsart [317] obtained an 18 per cent increase in tomatoes and a 28 per cent one in blackcurrants by soil application of manganese. Scharer and Werner [253] got similar results with vegetables, as can be seen in Table 15, but once again *too high levels* of manganese result in a decrease in vitamin content. Deficiency of molybdenum results in a marked decrease in vitamin C content of tomatoes and vegetables, but injections of molybdenum doubled the vitamin C content of the deficient plants in 3–5 days. As mentioned [178] (page 68), boron depresses this vitamin in turnip greens.

This relationship between trace elements and vitamins is not confined to plants, but less is known about it in animals. The synthesis of vitamin C in rat livers, for instance, is promoted by manganese. Cobalt is an integral part of vitamin B₁₂, which is so necessary for normal blood development, and selenium may be in some way associated with vitamin E, as will be apparent from Chapter VIII.

Amino acids

Another aspect of biological quality that is of very great importance is the amino-acid content of foods. Human beings as well as animals have a very definite amino-acid requirement. The body cannot synthesize certain of these amino acids from raw materials, and is thus dependent upon outside sources for these *essential* amino acids.*

Proteins and their breakdown products are the main source of amino acids, as they are composed of chains of various amino acids linked together. Hence, if a protein is relatively poor in an essential amino acid, as the maize protein zein is in lysine, or many legume proteins are in methionine, its nutritional value must be low. As a

* The known *essential* amino acids for adult humans are: lysine, tryptophane, methionine, (cystine), threonine, phenylalanine, (tyrosine), valine, leucine and isoleucine. In children histidine is also essential. The amino acids in brackets can be partially replaced by the ones preceding them.

Absence of essential amino acids in the diet leads to serious abnormalities.

The requirements of various species of animals are different from those of humans.

result of this, there is a very wide range in the food value of different proteins.

It has been shown conclusively that fertilizers do influence both the protein content and the protein composition in plants. It is also known that the trace elements do play an important part in determining the amino-acid composition in plant proteins, but there is still too little information to be able to get a clear insight into how significant trace elements are in this respect. This lack of information is surprising if we consider how widespread diseases due to amino-acid imbalances are in both humans and animals, but it must be appreciated that adequate analytical techniques to study these problems have only been developed recently.

Kochler and Albrecht [155], in a very informative experiment, clearly demonstrated the enhanced nutritional value of lucerne fertilized with trace elements and magnesium by feeding this to rabbits. The application of trace elements, or of magnesium on its own, did not improve the nutritional quality as measured by weight gains of rabbits, but when combined they gave an increase of about 15 per cent, as can be seen in Table 16. The treatment resulted in an increase of both tryptophane and methionine, the latter an essential amino acid somewhat low in legume proteins. In other experiments it was demonstrated that rabbits could differentiate between fertilized and unfertilized vegetation, and preferred fodder that had received trace-element applications. This information is no surprise to field experimentalists, who have known for a long time that rabbits and other

TABLE 16

The influence of trace-element fertilization of the soil on the nitrogen, tryptophane, and methionine content of lucerne, and the average weight gains of rabbits fed on it. From KOEHLER AND ALBRECHT [155]

SOIL		TRYPTO- PHANE, MG./G.	METHIO- NINE, MG./G.	AVERAGE GAIN IN WEIGHT OF RABBITS,
TREATMENT	N, %			G.
None	3.12	1.86	4.57	740
Mg alone	3.20	—	—	744
T.E. alone	3.19	—	4.20	699
Mg + T.E.	3.05	2.52	5.44	849

The application of magnesium (Mg) or of trace elements (T.E.) either separately or together to the soil does not alter the nitrogen content of lucerne appreciably, but the application of the two together markedly enhances the food value, as can be seen from the gain in weight of the rabbits. The content of the essential amino acids tryptophane and methionine is also significantly increased by the fertilizer treatment. The trace elements used were manganese, copper, boron, zinc, and cobalt.

animals can discriminate between vegetation receiving different fertilizer treatments and who tend to feed on the best nourished vegetation.

The above investigations were made with a mixture of trace elements. It can be shown that the tryptophane content of lucerne is proportional to boron levels in nutrient solutions (Table 17), while

TABLE 17

Boron concentration as a factor determining tryptophane content in lucerne. From SHELDON [277]

BORON IN PARTS PER 1,000 OF THE NUTRIENT SOLUTION	TRYPTOPHANE, P.P.M. DRY MATTER
0.00	1.27
0.22	1.36
0.44	2.17
1.08	2.55

The essential amino acid tryptophane's concentration in lucerne was clearly correlated to the boron concentration of the nutrient solution.

Vlasiuk [312] has shown that manganese fertilizers in the field diminish by 15 per cent the undesirable protein zein in maize and increase the other proteins. The content of tryptophane and of lysine, essential elements for man and farm animals, is increased at the same time. A more detailed account of the known trace element-amino-acid relationship is given by Schütte and Schendel [268]. Certainly the modern work substantiates completely the findings of the earlier investigators [120].

Wool

Wool is another protein that is influenced by trace elements. It is a commodity in which quality is of great economic importance. Good-quality wool, which is woven into quality textiles, must have a good 'crimp', that is to say, it must be very wavy. Some poor-quality wools lack this 'crimp', and the strands are more or less straight, often being brittle as well. This 'steely' wool is of very little value, and of poor quality, as it does not spin or weave well, and is too brittle to give the elastic thread that is so characteristic of wool.

Copper deficiency in the diet results in 'steely' wool, and the mechanism whereby copper is involved in wool formation is fairly well understood [102, 179, 295]. Wool is composed of strands of the protein keratin, which is built up of amino acids including the sulphur-containing cystein. This amino acid possesses a sulphydryl group -SH. In good crimpy wool the keratin molecules are linked by a cystein bridge where two sulphydryl groups join to form a disulphide

S S link. This junction is under the control of a copper-containing enzyme, and in its absence the keratin molecules do not form this bridge and the wool lacks crimp. This cross bonding is obviously of prime importance to quality.

Deficient sheep respond extremely rapidly to copper therapy, and within 24 hours of receiving copper they start to produce 'crimpy' wool. This striking result is clearly shown in Fig. 28, where the abrupt change from 'steely' wool in the deficient sheep (A) gives rise to wool of good quality (B) as soon as copper is fed.

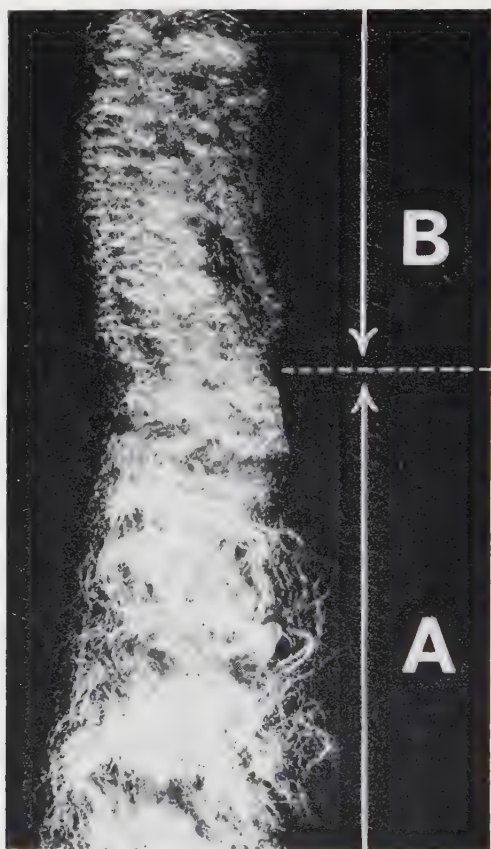


FIG. 28

AUTHOR'S PHOTOGRAPH

Wool quality and copper

Copper deficiency in sheep gives rise to poor-quality 'steely' wool. This wool is coarse and has very little 'crimp' (A), i.e. the tight wavy pattern that characterizes good wool. Within 24 hours of being supplied with adequate copper the nature of the wool changes, and good 'crimpy' wool is again produced (B).

Fat

In animals, such as pigs produced for meat, body weight is not the only important factor. A fat pig gives fatty bacon and ham, which is not popular with housewives. A good bacon is a lean one. Hence in pig breeding, while large and heavy animals are desirable, fat ones are not, as they give rise to unpopular and hence low-quality produce.

It is of interest that manganese deficiency does, in fact, give rise to *fat pigs*. The animals are excessively fat, have reduced body length and height, and shorter legs than comparable animals fed manganese at a level of 40 p.p.m. in their diet [225], as is clearly shown in the photographs in Fig. 29. The lean animals have a higher market value.

Certainly the trace elements play an important part in phospholipid and phosphatid synthesis. In rats a copper deficiency results in a considerable diminution of both of these compounds, as the lack of copper prevents the condensation of acyl Co·A with α -glycerophosphate to form phosphatidic acid [106]. The part played by vanadium in cholesterol synthesis is discussed in Chapter VIII.

Goitrogenic milk

Milk is an extremely important foodstuff and has been much studied. The diet of the cow is reflected not only in the quantity of milk that she produces but also in its quality. Butterfat, vitamin, and mineral content are readily influenced. Even the fertilization of the pastures with phosphatic and nitrogeous compounds alters the biological quality of the milk appreciably [3].

In Tasmania children consuming milk obtained from cows fed on marrow-stem kale (*chou mollier*), a plant belonging to the cabbage family, developed goitre, while other children did not. It has been known for a considerable length of time that plants of the cabbage family contain *goitrogenic* substances which can induce goitre if the level of iodine in the diet is not fairly high. The goitrogens were passing through the cow into the milk and increasing the children's iodine requirement and making them goitre prone. If the children received iodine supplement they could consume the milk without any harmful consequences [119].

But iodine fertilization can increase the iodine content of the milk, and so counteract the baneful effect of the goitrogens. As long ago as 1933, Seharrer *et al.* [252] showed that iodine is rapidly taken up by vegetation, and that cows grazing on iodine-rich pastures produced milk rich in this element. This appearance of iodine in the milk may be important in areas like the north of Scotland where radioactive iodine fall-out occurs. It may be concentrated in the milk.



FIG. 29

PLUMLEE *et al.* [225]

The influence of manganese on the development of excess fat in pigs. The pig (top) was raised on a diet containing 40 p.p.m. manganese. Its weight was 164 lb. at 132 days. Its litter mate (lower) had a diet containing only 0.5 p.p.m. manganese. Its weight was 174 lb. at 132 days: note its excessive fatness, and that the body and legs are shorter.

Recent experimental work has shown that various types of fertilizers can influence the goitrogenicity of kale very significantly. The vegetables were tested by feeding to rabbits and the weight of the

TABLE 18

Effects of various fertilizer treatments upon the goitrogenicity of kale (Brassica oleracea). From ALLCROFT AND SALT [1]

FERTILIZER TREATMENT OF KALE	WEIGHTS OF THYROID GLANDS, YOUNG RABBITS MG. FRESH TISSUE	
	1-4 DAYS OLD	5-9 DAYS OLD
(1) Control	11.7 $\pm$ 0.80	25.0 $\pm$ 15.9
(2) Nitro-chalk (Calcium ammonium nitrate)	8.8 $\pm$ 0.75	19.6 $\pm$ 5.24
(3) Chilean nitrate of soda	16.8 $\pm$ 1.87	48.8 $\pm$ 16.8
(4) Ammonium sulphate	11.6 $\pm$ 0.58	14.7 $\pm$ 3.76
(5) Ammonium sulphate + iodate (24 lb. KIO ₃ /acre)	11.0 $\pm$ 2.02	11.2 $\pm$ 1.91

Thyroid weight is taken as an index of the severity of goitre. It is quite clear that all the kales except those in treatment (5) are goitrogenic. Except for Chilean nitrate of soda, which produces a very marked increase in the goitrogenicity of the kale, none of the fertilizer treatments had a very marked influence. However, the addition of potassium iodate [5] to the soil either prevented the formation of the goitrogenic matter or made it ineffective, because rabbits fed this kale have completely normal thyroid glands.

thyroid gland was taken as an index of goitrogenetic activity. The results are shown in Table 18, and it is clear that the application of iodates removes the detrimental effect of the kale on the thyroid [1].

Egg quality

Eggs are fragile structures. This makes their transport and handling expensive. The less breakable a hen's egg is, the better, and this characteristic is determined, among other things, by the level of manganese in the diet of the parent hen. The breaking strength of eggs rose from an average of 6.6 lb. when the hens had 6.5 p.p.m. manganese in their feed to give an average of 9.3 lb. at 100 p.p.m. manganese [62]. Calcium and phosphorus in the diet antagonizes the manganese in the diet and increases the animal's requirement for the latter.

Manganese is found mainly in the yolk. Like many embryonic structures, the egg accumulates high levels of trace elements, and these often reflect the parental diet. Table 19 shows how the manganese content of egg yolks is very roughly proportional to its content

in the feed of the hens [107], and in this experiment there is an eight-fold variation in manganese levels.

TABLE 19

The relationship between the manganese content of the hen's diet and the manganese content of the egg yolk. From GALLUP AND NORRIS [107]

AGE OF HENS. WEEKS	MANGANESE, OF DIET, P.P.M.	MANGANESE, MG. 100 G. DRY YOLK
45	13	0.045
	200	0.152
24	53	0.108
	500	0.203
	1,000	0.342

Only traces of manganese were found in the egg white and shell.

The manganese content of the egg can be controlled by adjusting the level of manganese in the diet of the hen.

The hatchability of eggs is obviously of importance in chick raising, and iodine has been found to play an important part in determining this quality. Deficiency of this element results in an increase in the length of incubation and also in a large increase in the number of dead embryos, but not in a marked alteration of the fertility rate [246]. This is clearly shown in Table 20, which shows that in the

TABLE 20

Iodine deficiency as a factor in determining the length of incubation and hatchability of hen eggs. From ROGLER, PARKER, ANDREWS, AND CARRICK [246]

	CONTROL	IODINE DEFICIENT
Total number of fertile eggs	106	110
Total number of dead embryos	16	65
Chickens hatched on:		
21st day	90	0
22nd day	—	4
23rd day	—	9
24th day	—	14
25th day	—	11
26th day	—	6
TOTAL	90	44

Iodine deficiency results in a marked prolongation of the incubation period and a large increase in the number of dead embryos, as well as a pronounced decrease in hatchability.

iodine supplemented control group nearly 90 per cent of the eggs hatched on the twenty-first day, while none of the 40 per cent of the deficient eggs that hatched did so until later. The iodine content of

eggs can vary considerably, and it is governed to a large extent by the level of iodine in the diet. In six-month-old hens a range of iodine from 6.5 μg . I_2 /per egg* (hens diet contained 1.7 μg . I_2 /kg. feed) to 339 μg . I_2 /per egg (hens diet contained 56.3 μg . I_2 /kg. feed) [247], a fifty-fold variation in iodine content due to the nature of the feeding stuffs supplied. This is of importance not because of hatchability but also because eggs are an important item in human diets, and humans are prone to thyroid diseases.

Fermentations

In crops such as tea the weight of the harvest is not the only consideration, as the leaves have to be fermented before they give rise to the product that is sold commercially as tea. And the fermentation is of the utmost importance in determining the quality of the tea produced. Work in Ceylon has shown that the fermentation of black tea does not proceed properly unless the tea leaves contain at least 15 p.p.m. copper. This element is necessary because it is a constituent of the polyphenol oxidase that plays a critical part in the fermentation. In its absence no normal fermentation takes place. Yet this enzyme is not necessary for normal metabolic processes in the tea plant. Plants containing less than 10 p.p.m. copper can be both vigorous in growth and high yielders of leaves [232].

In cocoa the fermentation of the seeds of the cocoa tree is also important in determining their quality. Here again it appears that a copper-containing phenol oxidase plays an important part, as do some of the other trace elements, but accurate information does not exist yet [129].

Alkaloids

As trace elements influence the metabolism of plants, it is not surprising that they can also regulate the production of such things as alkaloids, compounds that may be regarded as the by-products of slightly unusual cellular processes. In fact, the concentration of one of these alkaloids, nicotine, has been found to be very closely linked to soil boron levels in tobacco plants. As is clear from Fig. 30, boron deficiency results in over a five-fold increase in nicotine content. The other trace elements, either in deficiency or excess, do not appear to influence the nicotine concentration considerably [284, 305]. As boron deficiency is common in some tobacco-growing areas, it is probable that the nicotine content of commercial tobacco must vary very considerably.

* Contents calculated per standard 60-g. egg.

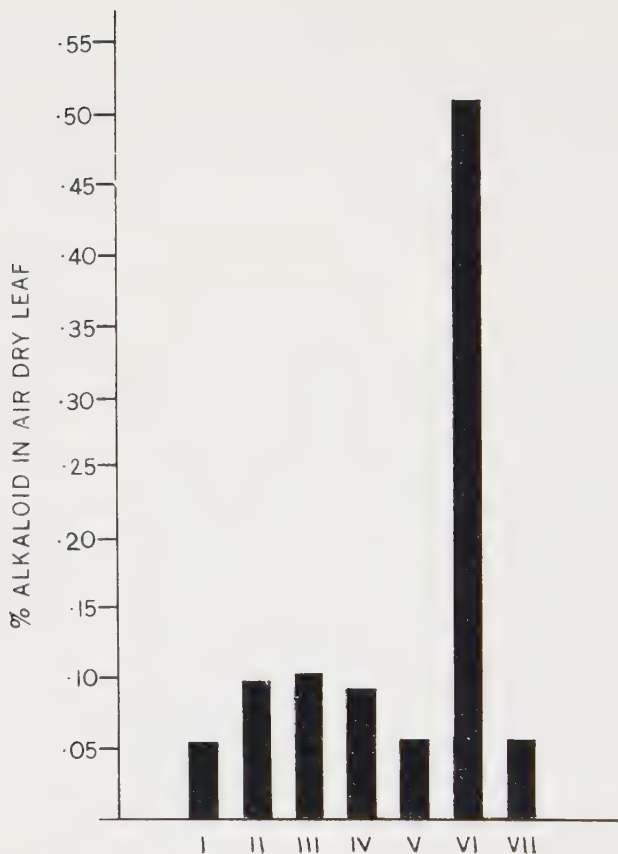


FIG. 30

AFTER STEINBERG AND JEFFRIES [284]

The influence of trace element deficiency upon the alkaloid content of tobacco leaves

- | | |
|-----------------------|----------------------------|
| I. Control. | V. Manganese deficient. |
| II. Iron deficient. | VI. Boron deficient. |
| III. Zinc deficient. | VII. Molybdenum deficient. |
| IV. Copper deficient. | |

Plants were Connecticut Broadleaf Tobacco grown in water culture. Boron deficiency results in a marked increase in the nicotine and other alkaloids in the leaf, but the deficiencies of the other elements have only a slight influence upon the concentration of leaf alkaloids.

Other criteria

These few examples must suffice to demonstrate the functions that trace elements can exercise in determining quality. But as quality concerns such a vast and varied number of criteria, it is quite clear

that this concept must appear again and again in these pages. The influence that boron has on celery fibres, or copper on the lodging of wheat, that fluorine has on healthy teeth and iodine on reproductive cycles, these and other phenomena discussed subsequently all influence *quality*, a very important characteristic.

VI | Normal and abnormal anatomy associated with trace elements

The term trace element can be misleading, at times. It may give the impression that these elements are not as important as the major elements. Nowhere is the falsity of this impression more obviously evident than in a study of the anatomical implications of these minor elements. As they are *essential*, their absence or presence exert a tremendous influence; just as great as that of the major elements. It must be stressed that *trace* or *minor* only refers to the concentrations at which they are effective and not to their effect. This can be very clearly seen in the marked anatomical variations caused by or linked to pathological conditions associated with trace element imbalance.

Balanced nutrition

Nutrition is known to have a very pronounced influence upon the appearance and behaviour of man, animals, and plants. Populations living on sparse diets may be small, while those living on more plentiful diets are larger. This is as true for the Japanese children living in California, who are several inches taller than their counterparts in Japan, as it is for the small but perfectly formed geranium growing on a wall, whose well-fertilized parent is a large garden bush. *Balanced nutrition* can occur at various levels, and sparse diets are not necessarily deficient ones, although they often are. Fig. 31 shows two healthy plants, quite different in size due to differences in the level of nutrition. They are healthy and normal because their nutrition is balanced. This is a very important point to grasp, as Chapter XII will show that unbalanced nutrition can be found in relatively high-yielding crops and be induced by faulty fertilizer practice.

Plant abnormalities

Whip-Tail in cauliflower. A very fine example of growth abnormality is found in the molybdenum-deficient cauliflower, which shows 'whip-tail', cf. Fig. 8 (page 38). In this condition normal leaves are not formed and the lamina or leaf-blade is virtually absent, leaving only the mid-rib present. If the condition is very acute the growing points die completely. This completely abnormal growth is due to a lack of only a few p.p.m. of molybdenum in the soil, and it can usually be



FIG. 31

The influence of different levels of balanced nutrition on the size of two healthy plants

Two healthy and normal plants of *Dimorphanthera* sp., the small one on the left grown in sand, the right-hand one in a good garden loam. Both were planted at the same time.

The plant grown on sand is small, as its supply of nutrients was limited; the loam-grown one is large, as it had a good supply of nutrients. *Both are healthy and normal because they have had a balanced supply of nutrients.* Health and normality can be present without optimal development having taken place.

cured by the application of less than 1 lb. of sodium molybdate per acre.

Zinc deficiencies

Zinc deficiencies also result in very abnormal growth. 'Little leaf' and 'rosette' are conditions found in both deciduous and citrus fruit trees, as a result of zinc deficiency. The shoots of the trees give rise to

very small pointed leaves, in contrast to large flat ones as is shown in Fig. 32. If the internodal growth of the shoots is severely restricted the leaves then cluster around the stem to give a typical rosette appearance. The leaves themselves are usually chlorotic, i.e. an uneven green colour instead of a normal healthy deep green, and this lack of chlorophyll results in a further yield diminution.

As well as altering the leaf size, zinc deficiency also alters the nature of the leaf anatomy considerably, and the number of stomata* is greatly increased in these leaves, although many are quite abnormal

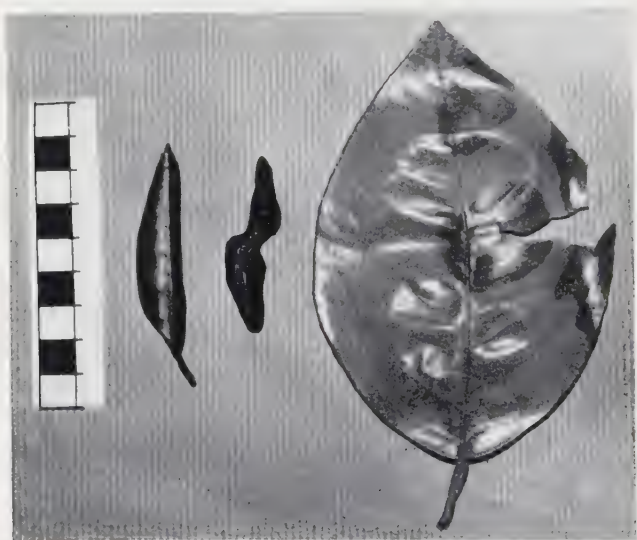


FIG. 32

Examples of zinc deficiency in citrus leaves, showing the characteristic 'little leaf' condition

The large leaf on the right is not quite normal in shape, but almost normal in size. In citrus in South Africa zinc deficiency can result in characteristic chlorotic patterns on the leaves, as well as bringing about abnormal growth, resulting in small and narrow leaves.

and non-functional (cf. Fig. 33). These anatomical alterations obviously result in a marked alteration of the water physiology of the leaves, cf. Chapter X.

In flax, where long fibres are commercially very desirable, zinc deficiency results in an alteration of growth habit. Instead of straight single shoots, bushy branching plants are produced, which are of little value [63].

* Pores in the epidermis of plants, especially in the leaves, which are present in large numbers and through which diffusion of gases, especially water vapour, takes place.

Boron deficiencies

If marked growth abnormalities occur in plants, then it is very likely that meristematic tissues are involved, as these produce the new cells. This is probably the case, as in many deficiencies the terminal buds where the meristems* are situated, die, and result in the condition known as 'die back'. In some of these cases lateral buds develop to a limited extent, giving rise to a shrubby development. But except in the case of boron deficiencies, very little is known of the anatomical changes taking place in or near the meristems.

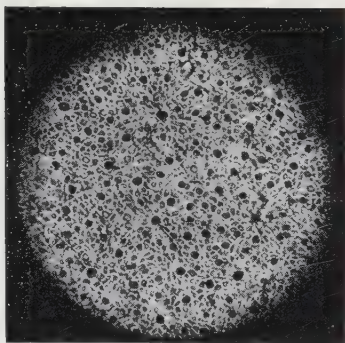
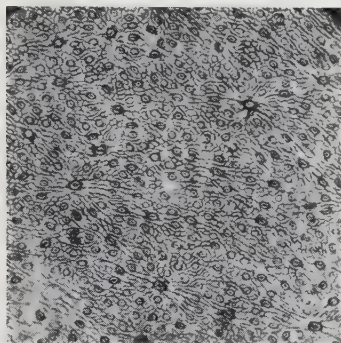


FIG. 33

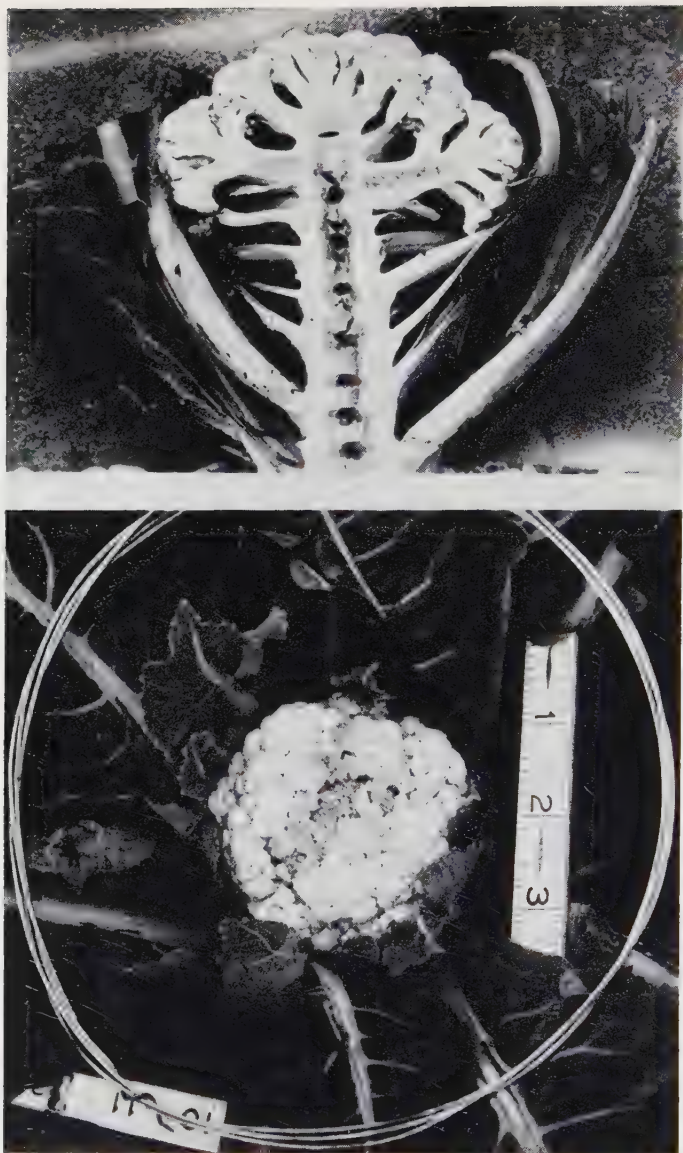
Zinc deficiency in citrus results in abnormal development of the leaf tissues

The photograph on the left shows the distribution of stomata (pores) on a normal leaf. The one on the right shows the large number of stomata found in zinc-deficient leaves, most of them being non-functional. Stomatal development is seriously upset. (Both photographs $\times 120$.)

In celery, a plant with very pronounced boron requirements, a deficiency results in abnormal cells developing just behind the true meristems. These cells tend to be larger than normal, and they collapse and die, giving rise to the so-called necrotic or dead zones just behind the growing tips. In sugar beet, these necrotic regions are extensive and give rise to the characteristic 'crown rot', which forms a large necrotic hollow region inside the stem and root. In other plants, such as the cauliflower and its relatives, this hollow region may be formed at a distance from the meristem, as is depicted in Fig. 34.

This post-meristematic damage can clearly result in gross malfor-

* Localized regions of active cell division from which permanent tissues are derived.



Photos by courtesy of Borax Consolidated Ltd

FIG. 34

Cauliflowers showing typical boron deficiency

In many plants lack of boron results in the breakdown of certain tissues. In this example a boron deficiency in cauliflower has resulted in a characteristic breakdown of the central stem tissue and (below) browning.

mations of such structures as leaves, roots, and fruits. As the deficiencies do not influence the meristematic regions uniformly, growth from such regions cannot be uniform, and some parts of the tissue may develop relatively normally, resulting in very contorted growth and deformed roots, stems, and fruits, as seen in Figs. 9 (page 39) and 25 (page 63). In some plants, if the deficiency becomes too severe, the rooting habit is completely altered and large numbers of small lateral roots are produced instead of a normal tap-root system.

Cell alterations

These abnormalities resulting from boron deficiencies must be associated with marked alterations in the cells themselves. As Reed [235] has shown, the nature of the cell protoplasm is altered, and the presence of phenolic substances can be demonstrated. Eventually, large masses of flocculent material are aggregated into bodies known as coacervates, which are formed well before the cell dies (Fig. 35). Investigations of the olive plant show that phloem, the vascular tissue that conducts the bulk of the plant's organic nutrients, disintegrates rapidly when boron deficiency is experienced. Both the sieve tubes and the companion cells vacuolate prematurely and disrupt their contents; then they become filled with a dark viscous melanotic material which prevents the sieve tubes from carrying out their normal role in the translocation of sugars and amino acids.

TABLE 21

Cell wall thickness in normal and boron-deficient celery. After SPURR [282]

VARIETY OF CELERY	COLLENCHYMA WALL THICKNESS, μ		PHLOEM PARENCHYMA WALL THICKNESS, μ	
	0.50	0.00	0.50	0.00
	P.P.M.B. (NORMAL)	P.P.M.B. (DEFICIENT)	P.P.M.B. (NORMAL)	P.P.M.B. (DEFICIENT)
Dwarf Golden Self				
Blanching	8.2	2.8	1.0	3.5
Utah 15	8.0	2.3	0.81	2.2
Utah 10 B	9.4	4.1	1.2	2.4

In boron-deficient celery the cell structure of both the collenchyma and the parenchyma of the edible petiole is altered. The thick-walled collenchyma cells which form a mechanical supporting tissue in the petiole have their wall thickening much reduced in deficient plants. On the other hand, the paranchyma cell walls become thickened. Some of Spurr's [282] measurements given in Table 21 show this very clearly.

In cotton plants boron deficiency results in the expected necrosis of the apical regions. But as well as this, it induces the premature

formation of an abscission layer resulting in a marked increase of boll-shedding,* to the great economic detriment of the grower [209].

In cereals, plants which have a very low boron requirement, the deficiency of this element results in abnormalities developing in the sporogenous tissues, while the rest of the cells remain normal. The nuclei of the cells are damaged, and cell division is impeded. Although the cells assume abnormal shapes, the nature of the walls does not appear to be altered [171]. In maize lack of boron results in ears

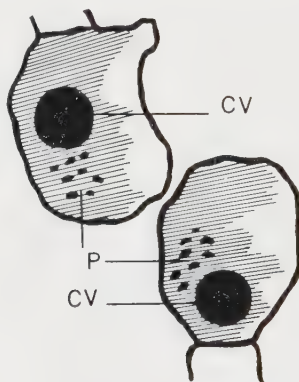


FIG. 35

AFTER REED [235]

Cytological alterations due to boron deficiencies

CV = Coacervates.

P = Phenolic material which filled the vacuoles and obscured other cell contents.

Deficiencies also result in marked alteration of the cell contents, some of which are of cytological importance. This drawing shows the presence of large coacervates (aggregates of pyridoxin enveloped with lipid material) in leaf cells of boron-deficient olives. These are very abnormal, and do not occur in healthy cells.

without either stamens or stigma [9]. Other reproductive structures have a need for boron. Many pollen grains will not germinate and develop pollen tubes in the absence of boron. This phenomenon was not appreciated for a long time by botanists trying to germinate pollen on synthetic media in laboratories.

Lodging in cereals

Lodging, i.e. plants lying on the ground instead of standing vertically, is a condition in cereals that causes great economic loss, as it makes harvesting difficult, if not impossible, and if the soil is wet, promotes

* Increase in the amount of cotton fruits shed before harvesting.

fungal infection. If the anatomical structure of the plant is mechanically weak, then it is prone to lodging. Hence the investigations which show that copper and manganese prevent lodging and are of real practical consequence in agriculture.

Russian scientists [226] have shown that copper influences the vascular anatomy of oats. Increasing the copper content gives rise to a vascular distribution that tends to prevent lodging. This was especially true when nitrogenous fertilizers were used. These have long been known to make cereals susceptible to lodging, and have been

TABLE 22

The influence of fertilizers, with and without copper, on the lodging and physical characteristics of oats. From POLUKHJA AND MASLINANAJA [226]

FERTILIZERS	GROWING STEM			MATURE STEM		
	PK	N + PK	NPK + Cu	PK	N + PK	NPK + Cu
	LODG- ING	LODG- ING	LODG- ING	LODG- ING	LODG- ING	LODG- ING
Thickness of the epidermal cell wall:						
Microns	0.47	0.41	0.97	2.43	3.12	4.68
Relative	100	87.2	206.1	100	116.7	191.5
Thickness of hypodermal layer of Schlerenchyma:						
Microns	1.59	0.61	3.49	5.11	7.14	7.23
Relative	100	38.5	219.1	100	139.8	141.5
Diameter of 3rd internode:						
Cm.	3.03	3.96	3.92	3.10	3.70	4.57
Relative	100	130.8	129.4	100	119.3	147.5
Average height of plants:						
Cm.	—	—	—	114	123	144
Relative	—	—	—	100	107.8	126.3
Ratio:						
$\frac{\text{Height of plant}}{\text{Diameter of 3rd internode}} \times 10$	—	—	—	368	333	315
$\frac{\text{Height of plant}}{\text{Thickness of epidermal wall}} \times 100$	—	—	—	469	394	308

All fertilizer treatments lacking copper gave rise to lodgings, but the nitrogen ones gave it most acutely. The presence of copper doubles the thickness of both the epidermal wall and of the hypodermal schlerenchyma belt in young plants and very appreciably in older ones. While nitrogen increases the diameter of the third internode, in the presence of copper in mature plants there is an even greater increase.

The use of copper also gives the tallest plants, but in spite of this, as the two ratios indicate, the copper-receiving plants are mechanically the most stable.

shown to increase the plant's copper requirements (see page 181). As a consequence of the copper treatment, the thickness of the epidermal cell walls, the hypodermal layers of schlerenchyma, and the diameter of the nodes were all increased, with a resulting resistance to lodging. Some details are given in Table 22.

Kannenber [146] reports similar results of oats and rye responding

to copper treatment. As well as causing lodging, lack of copper gives lower yields of grain, as it causes 'blind ears'. Not only the cereals but also grasses and clovers suffer from soft and pappy stalks in the absence of adequate copper. The beneficial consequences of copper therapy are clearly seen in the photograph in Fig. 36.



FIG. 36

KANNENBERG [146]

Lodging in cereals

Mature plants of German fodder grass grown in pots in the open. Those on the left received no copper and, as well as lodging, these plants had 'blind' ears, lacking grain. On the right the plants receiving copper developed normally, with erect stems and 'full' ears containing fertile grain.

Roach [240] has observed that the application of manganese prevents the formation of wheat prone to lodging. The mechanical weakness that leads to this tendency to lie flat on the ground must have been overcome by the manganese, but no anatomical details were recorded.

Abnormalities in man and animals

While the anatomical implications of trace-element imbalance in plants are interesting, in humans and animals they are truly remarkable. This stems from the fact that human and animal conditions are more readily observed and their implication more easily compre-

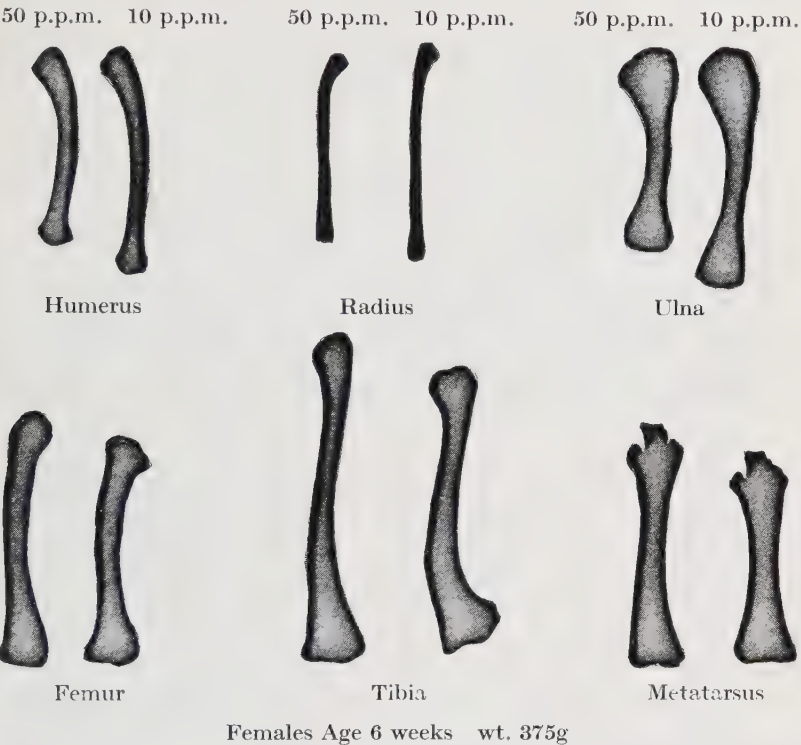


FIG. 37 FROM CASKEY AND NORRIS [62]

Influence of manganese deficiency on bone development in chicks

The bones on the deficient diet (10 p.p.m.) show a pronounced shortening and thickening.

hended by the casual observer, while plant abnormalities are not so readily appreciated. Unfortunately, although imbalances in humans, and to a lesser extent animals, are of greater interest to most people, accurate information concerning the role of trace elements is not very plentiful, except perhaps in the case of iodine, and it is only recently that more detailed investigations have been undertaken.

Bone abnormalities

In animal physiology the trace element manganese plays a very important part in regulating bone development, and when it is deficient it leads to some very marked skeletal malformations. In poultry the disease termed perosis or slipped tendon [306], in which the Achilles tendon frequently slips out of place, and in which the animals in extreme cases walk on their hocks, is caused by lack of manganese. The limb bones are very abnormal, as is clear from Fig. 37. The tibia metatarsal bones thicken and bend, and the legs are

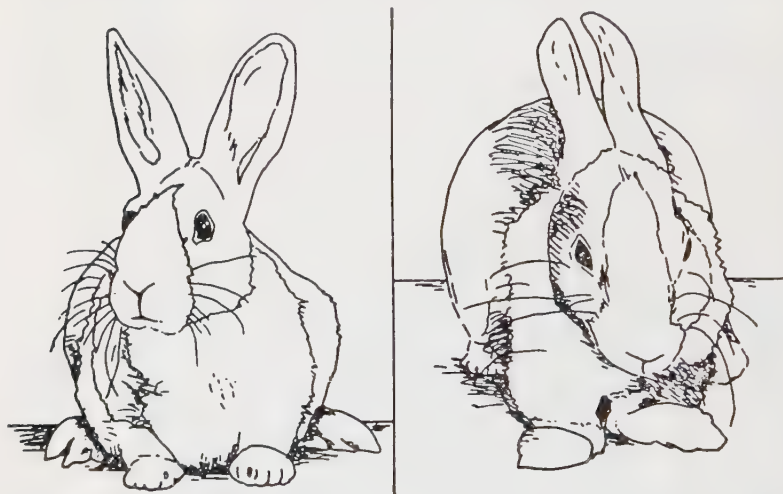


FIG. 38

UNDERWOOD [306]

Manganese deficiency in the rabbit

Manganese deficiency in rabbits results in gross deformity of the front legs. This can be clearly seen by comparing the deficient animal (*right*) with a normal rabbit (*left*).

malformed. This condition can be prevented by supplying adequate supplies of manganese. In rodents a similar condition resulting in malformation of the fore-legs is known, and is depicted in Fig. 38, where the rabbit on the right has the flat-footed appearance brought on by this deficiency. In Holland [116] a bone abnormality analogous to these conditions is found in children, but it has not yet been proved to be due to a disturbance of manganese metabolism. The alteration in pig skeletons has already been mentioned in Chapter V. In swine, manganese deficiency results in a decreased body length and height, shorter legs, and occasionally in bowed legs and enlarged joints, with definite shortening of the radius, ulna, tibia, and other

bones. Eowing is due to the closure of the distal epiphyseal plates of the radius, which stops its growth in length, while the ulna can continue to grow, resulting in malformation of the fore-legs [225].

Manganese deficiency leads to a reduced calcification of the bones and to many characteristic changes within the bones themselves. In dogs a very similar condition, in which the legs are very bowed and the flat-footed appearance is found, can be caused by copper de-



FIG. 39

BAXTER [27A]

The influence of copper on bone formation in dogs

Three litter-mate dogs after three months on purified diets. Dog (above left) is the normal control animal receiving copper in its diet. The other two lack copper. They have a rough coat and deformed legs.

In the rear view of a copper deficient dog (left) note the hyperextension of wrist joints. This dog developed peculiar sacculations over the ischial tuberosity.



ficiency. Some examples are shown in Fig. 39. This condition, when examined more closely, shows that the bone abnormalities are due to a very different cause [27A]. Again, there is reduced calcification of the bones, but in this case, while bone calcification occurs at the normal rate, the reabsorption of the calcium is excessive and leads to this condition. Both manganese and copper deficiency lead to reduced calcium in the bones, but for completely different physiological reasons [306].

Elements such as vanadium and strontium have been recorded as

having a marked stimulatory influence upon the mineralization of bones and teeth in animals. The incidence of caries was higher in animals deficient in these elements than in controls [203]. While strontium is not generally regarded as an essential element, there is much evidence that would indicate that vanadium should be so regarded, as it plays a significant part in many important processes.

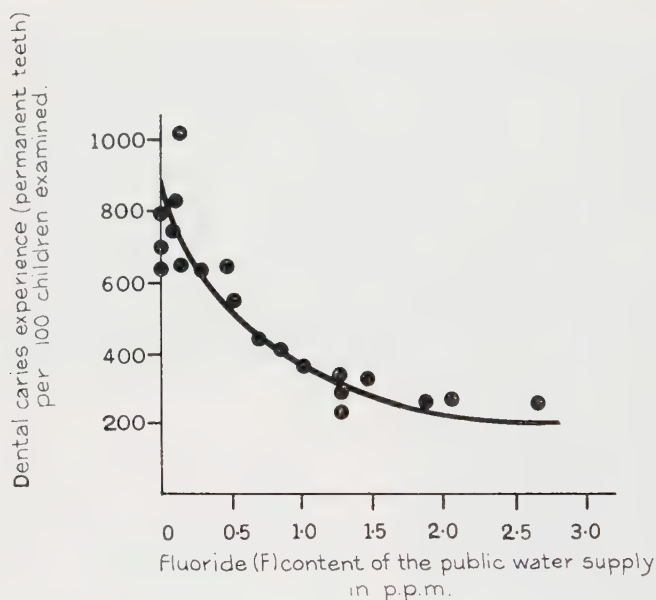


FIG. 40

MCLURE [178A]

The relationship between dental caries and the fluorine content of the public water supply

The relationship between the amount of dental caries of permanent teeth observed in 7,257 selected 12-14-year-old white schoolchildren of twenty-one cities of four states in the U.S.A., and the fluoride (F) content of the public water supply.

The role of fluorine in bone and tooth formation is very great. That there is a relationship between the absence of fluorine content and the incidence of caries is fairly well established. In Fig. 40 a graph depicting the incidence of caries at various levels of fluorine in the public water supply shows a clear relationship between the presence of fluorine and healthy teeth. It is but a step further to suggest that in areas where the incidence of caries is high due to lack of fluorine water supplies should be fluorinated as a therapeutic measure. This has been done in some areas, but it is strongly opposed

in others, as fluorine is a very toxic substance, and the limits of safety are very small, as we shall see later.

It has been shown in rats that molybdenum reduces the incidence of caries in experimental rats [288, 289], and that it enhances the effect of fluorine in preventing this condition in teeth, as can be seen from Table 23. The observation made in New Zealand that children

TABLE 23

The synergistic effect of molybdenum and fluorine on dental caries in rats.
From STOOKEY et al. [289]

ADDITION TO DRINKING WATER OF	REDUCED CARIES BY
25 p.p.m. molybdenum	18%
25 p.p.m. fluorine	32%
25 p.p.m. Mo + 25 p.p.m. F	52%

Both the molybdenum and the fluorine separately decrease the incidence of caries. When supplied together, the beneficial effect is greatly enhanced.

eating vegetables from the molybdenum-rich Napier area had fewer caries than those fed on vegetables from other areas, such as Hastings, is in complete agreement with these findings. The rat experiments used to investigate this state of affairs indicate the importance of molybdenum [176] in the development of healthy teeth.

Fluorine is a very toxic element, as has been mentioned, and fluorine toxicity or fluorosis is found in many parts of the world, where both stock and man are affected. In mild cases excess fluorine leads to a characteristic mottling of the teeth, which tends to be more unsightly than dangerous, as the teeth themselves remain functional. The enamel is abnormally thin in places, pitted, and lacks the lustre and translucence of normal enamel. This condition is found in Mecca [17], as well as many other parts of the world where the fluorine content of the drinking water is high. Even in countries like Britain, widespread mottling of the teeth has been recorded. But in severe cases, usually found after years of living in a toxic area, grave bone malformations occur. In South India 'poker-back' is found [218, 279]. Excessive calcification of the backbone and ribs make bending and normal breathing impossible, see Fig. 41, and the rigid posture gives the name to this condition. New bones, or bony projections, are formed on the backbone and in the limbs, where bony outgrowths may be found on the radius and ulna. The symptoms of fluorosis may vary in different parts of the world for reasons that are not known. In South Africa severe fluorosis does not show 'poker-back' symptoms, although marked bone abnormalities, especially bowing of the femora and tibiae, are still the most prominent symptoms [89].



A



B

C

Nerve and muscular abnormalities

A number of conditions involving muscular or nerve abnormalities are known which are linked to trace-element imbalances. In swine iron deficiency was reported to result in a 50 per cent increase in heart weight relative to body weight, while copper deficiency resulted



FIG. 42 FROM 'SHEEP HUSBANDRY', BY A. FRASER AND J. H. STAMP
Swayback in lambs and calf

Deficiency of copper in the diet of either cattle or sheep results in animals which cannot co-ordinate the movement of their limbs properly. This results in a staggering gait, and usually in a swaying of the hind-quarters, which gives the name 'swayback' to this condition. This photograph shows characteristic gait of a lamb with swayback caused by a degeneration of the nervous system, resulting from copper deficiency.

in a 200 per cent increase. The other organs, such as liver and kidney, were influenced to a much lesser extent [121].

Copper deficiency is particularly associated with degeneration of the nervous system. Sway-back is a copper-deficiency disease found

FIG. 41

SHORTT ET AL. [279]

Fluorosis in man

Chronic excess of fluorine results in fluorosis. In parts of India endemic fluorosis results in 'poker-back', a condition where the backbone calcifies and cannot be bent, as this picture clearly shows. The ribs also calcify, making normal breathing impossible and forcing the sufferer to use abdominal breathing (A).

Note the calcification of the Achilles tendon in the ankle (B) and the bony outgrowths between the bones of the forearm (C).

in lambs and to a lesser extent in calves. The young animals have symptoms of a spastic paralysis which causes the animals to stagger and sway, as their co-ordination is extremely poor. In severe cases the hind-quarters are paralysed and the animals cannot rise, and soon die. This condition is usually coupled with anaemia and steely wool (cf. Fig. 42). The animals may be born like this, or develop this disease after several months. These animals are born to parents apparently healthy, but suffering from copper deficiency and grazing copper-deficient (either simple or induced) pastures [306].

Autopsy shows that this condition, with its lack of muscular co-ordination, is mainly due to myelin degeneration of the central nervous system. Myelin is the fatty insulation around the nerves. When this insulation breaks down the nervous impulses, which are electrical in nature, can no longer be efficiently conducted, and inco-ordination and eventually paralysis are the result. Severe brain lesions are also frequently present, and the normal healthy tissue of the brain is replaced by a pulpy mass of fluids, as is shown in Fig. 43 [316]. Both these kinds of degeneration result in severe ataxia or inco-ordination. One of the major constituents of the myelin complex are phospholipids, and Gallagher *et al.* [106] have shown how copper deficiency depresses their synthesis in rats. It is probable that the same mechanism is responsible for demyelination in lambs and calves.

Another copper-deficiency disease is reported from Australia. Falling Disease is a fatal condition in cattle [31]. The animals fall over and die of heart failure, quite suddenly. Death is the termination of a severe copper deficiency, and autopsy reveals glomerular lesions and myocardial fibrosis of the heart muscle. The lesions upset the circulation of the blood and lead to sudden heart failure. The condition is seasonal, and yet the copper deficiency is chronic. The extent of myocardial degeneration is not proportional to the incidence of Falling Disease. The entire explanation of this disease is obviously complex and still escapes us.

The fact that selenium is a very beneficial element and probably an essential one has already been made (Chapter IV), and that excess of it results in Alkali Disease, cf. Fig. 7, with its distressing foot malformation. Selenium is a component of Factor 3, a substance that prevents liver necrosis in rats [269]. A congenital condition in lambs termed White Muscle Disease was found to respond to selenium. In this disease muscular dystrophy results in white or greyish areas of degenerated muscle fibres, which are limited in extent and give white patches in the meat. This obviously impairs muscular function, and often results in lameness [19]. Injection of vitamin E (α -tocopherol) or of selenium prevent this condition, while ewes in late pregnancy, if injected with selenium, had a marked decrease in deaths caused by White Muscle Disease in their lambs [92]. While selenium is apparently

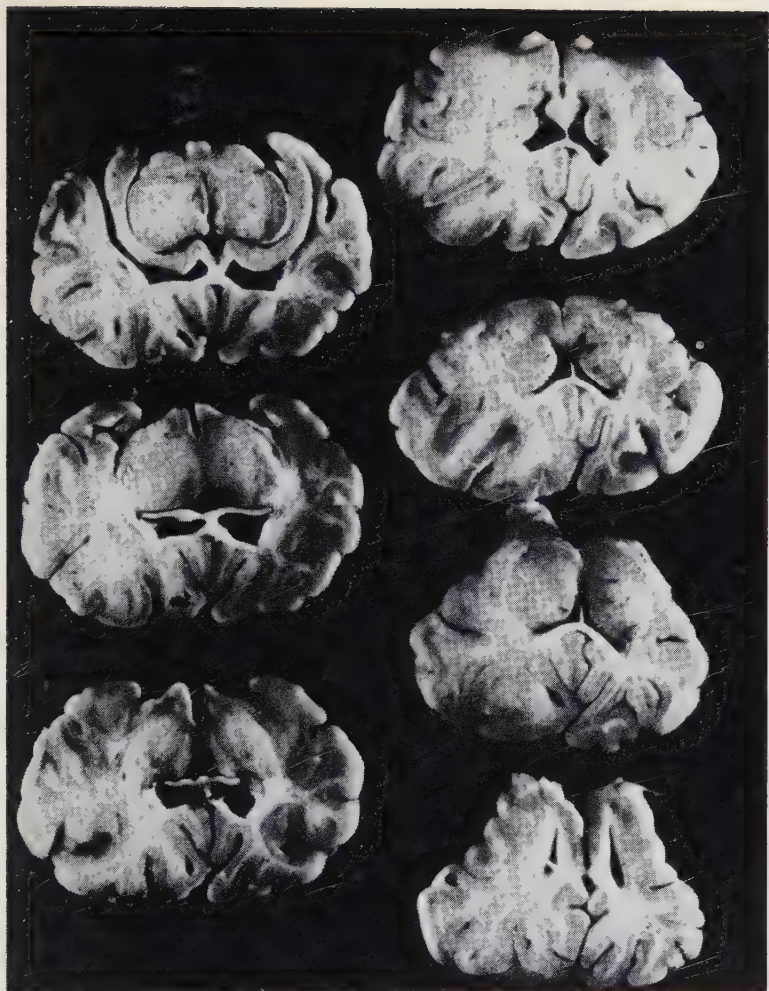


FIG. 43

VOISIN [316]

Brain lesions in copper-deficient lambs

The great extent of the brain lesions which can result in lambs from copper deficiency is shown in the autopsied brains shown above. The degeneration of this tissue leads to irreversible changes which result in lack of co-ordination, paralysis, and in severe cases, death.

a prophylactic as well as a therapeutic agent for this condition, its relationship to vitamin E, and the way in which it protects against a deficiency of this vitamin is not clear. Certainly in the rabbit other anti-oxidants beside sodium selenite can replace dietary α -tocopherol and prevent symptoms of serious vitamin E deficiency [21].

TABLE 24

Incidence of goitre in schoolchildren before and four years after the institution of prophylaxis with iodized baker's salt (39 mg. KI per kg. salt). From TERPSTRA AND QUERIDO [297]

	BEFORE PROPHYLAXIS			AFTER PROPHYLAXIS		
	NUMBER OF CHILDREN	GOITRE	GOITRE, %	NUMBER OF CHILDREN	GOITRE	GOITRE, %
Nursery School 1	57	18	31	67	2	3
Nursery School 2	49	29	58	63	4	6
Girls' School	116	62	53	131	25	19

The beneficial response to the iodine is very clear. It should be noted that not all the goitre is cured and that schoolgirls have a higher incidence of goitre than nursery schoolchildren, after iodine prophylaxis.

The swollen thyroid gland that gives rise to the big neck in goitre is known to most people (Fig. 6). This disease is usually caused by a deficiency of iodine, and if it is a case of simple iodine deficiency goitre it usually responds well to oral iodine therapy, such as the use of iodised salt. A fairly recent report from Holland shows the type of response obtained to iodine therapy (Table 24).



FIG. 44

GOITRE IN ANIMALS [12]

Typical case of severe goitre in an adult Angora goat

Animals suffer extensively from goitre. The symptoms are similar to those of human goitre. This adult Angora goat shows the typical 'big neck' condition.

Cellular changes precede the gross anatomical changes, and there is a decrease in stainable colloids in the cell vacuoles. As the gland increases in size the blood vessels become congested, and new vesicles are formed in the connective tissue. If the iodine shortage is acute,

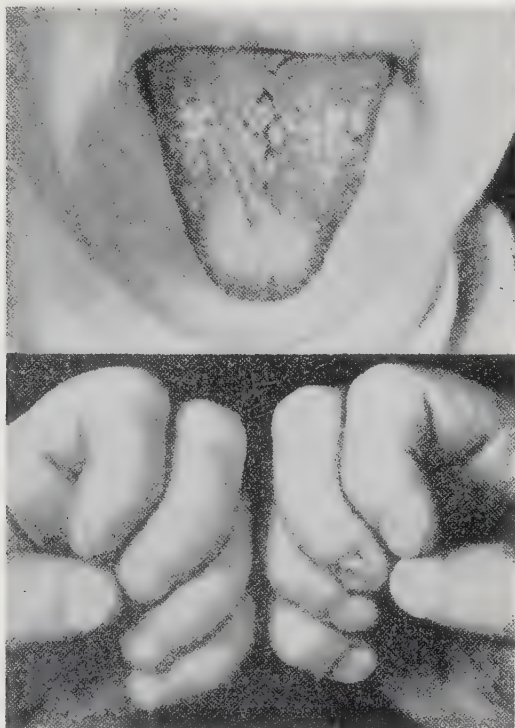


FIG. 45

Iron-deficiency anaemia in humans

DARBY [80A]

Some of the clinical symptoms of iron-deficiency anaemia as exhibited by a woman patient suffering from hypochromic microcytic anaemia, due to menorrhagia (an over-abundance of menstrual discharge). This type of anaemia used to be termed 'secondary anaemia' and is characterized by certain changes in the blood. In this case, inflammation of the tongue, inflammation of the corners of the mouth, and the hollow depressed, spoon-shaped nails are symptoms of this condition. All these cleared up with simple ferrous sulphate therapy.

then excessive multiplication of the follicular epithelium takes place. This layer gets folded over and over upon itself to give rise to the greatly enlarged, but functionless, gland of the goitre patient [297].

Goitre is very widespread among all kinds of animals, and can cause severe economic problems in parts of the world. In general, the symp-

toms are similar to those in humans, as can be seen in the photograph of the large swollen neck of a goitrous goat (Fig. 44).

It must not be supposed that all goitres are simple iodine deficiencies, for they are not. A more detailed discussion of goitre, its occurrence and causes, will be given in Chapter VIII, when health and disease will be discussed.

Anaemia

To conclude this chapter, reference will be made briefly to anaemia. It is not generally appreciated that iron-deficiency anaemia is responsible not only for the facial pallor it produces, but in severe cases also can bring about or be associated with the formation of spoon-shaped finger-nails, roughness of the tongue and cracking of the edge of the mouth, as shown in Fig. 45.

It is clear, from these examples cited here, that trace elements can and do play a great part in anatomical development. They show clearly how essential mineral elements are necessary for normal growth and normal functioning, as any imbalance results in abnormal development.

VII | The role of trace elements in reproduction and some other physiological processes

The micro elements play a very important part in reproductive processes in all forms of life, and the consequences of imbalances are truly spectacular. Right from the start, it must be remembered that the term reproduction covers a variety of phenomena, and that in lower organisms there are asexual as well as sexual methods of propagation. Even the sexual reproduction that is found in higher plants can only be compared with that of mammals in a very general way.

Micro-organisms and fungi

Simple bacteria and fungi require trace elements as well as other nutrients for their normal development. Bacteria have the simplest possible method of reproduction—simple fission into two daughter cells. The relative lack of any of the essential nutrients, be they trace or organic ones, results in this asexual process slowing up and fewer divisions taking place. This causes a much decreased rate of reproduction of the bacteria to take place.

The mould fungus *Aspergillus niger* is a test organism that has been studied intensively, and that is, in fact, used for microbiological assays of trace elements [200]. In assaying manganese, molybdenum, or zinc the weight of the resulting fungus is important, but in copper determinations the level of this element is assessed by the quantity and colour of asexual spores produced. At low levels of copper the spores are few and not pigmented. With increasing copper concentration the pigmentation of the spores increases, until in the normal mould dense black masses of spores are formed.

In the bacterium *Azobacter chroococcum* the normal black pigment does not develop in the absence of copper.

The unicellular green alga *Chlamydomonas* has a more complicated reproductive pattern than bacteria. It also shows sexual reproduction. Kuhn [162] has observed that the gametes (the reproductive cells), which normally are capable of functioning as either male or female, can be induced to function as purely male gametes if small amounts of boron are introduced into their surroundings. In the presence of boron these gametes behaved in a characteristically male manner and only fused with female ones.

Seed and fruit production

The important part that micro nutrients play in the reproduction of higher plants is often seen by the marked decrease in grain yields of cereals and fruit yields when deficiencies occur. For instance, in the manganese deficiency of oats called Gray Speck there are usually many 'blind heads', that is to say, flowers that have not set seeds. This lack of seed or fruit production is one of great economic implication. But unfortunately the exact mechanisms involved are not

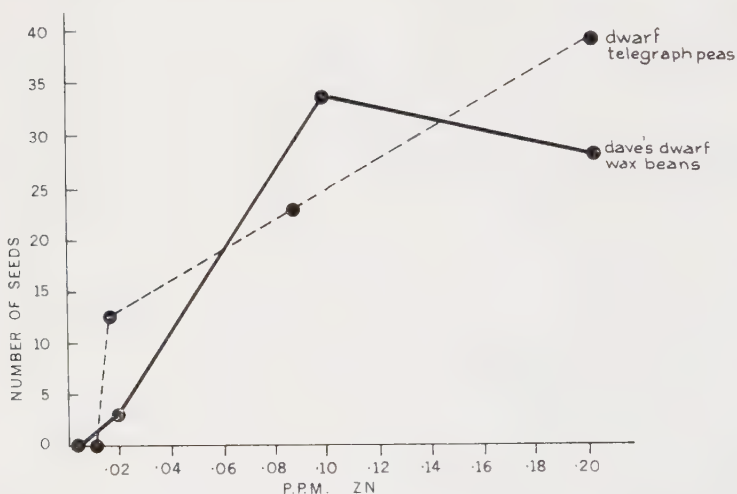


FIG. 46

AFTER REED [234]

The zinc requirement of peas and beans during the vegetative and reproductive phases of growth

Not all phases of a plant or animal's life have the same mineral requirements. The zinc requirement of vegetative peas and beans is much lower than their requirements during seed production. This graph shows clearly that the concentration of zinc in the nutrient solution has a marked influence upon seed production, even when the plants are apparently 'healthy'.

clear. Copper deficiency in many crops results in a marked reduction in generative development, although the vegetative growth need not be disturbed to anything like the same extent [228].

Clearly, reproduction can involve a variety of biochemical processes, any of which can result in the decreased production of viable seed or of fruit formation. Reed's work [234] makes it quite clear that much higher levels of zinc are required for seed production than for vegetative growth (Fig. 46).

It must be remembered that seed production is a quite distinct

phenomena from fruit formation. Seedless fruits, such as bananas, are well known, and others, such as seedless tomatoes, can readily be produced by means of hormone sprays. Fruit formation is not necessarily reproductive in nature, as it need not imply seed formation. Nor need seed formation imply viable seed production, as the percentage of viable seeds is much decreased in some trace-element deficiencies.

As far as the fertilization process is concerned, it has been known for a long time that boron is necessary for the proper germination of many pollens [111]. In the absence of boron the pollen tubes burst, or become so distorted that male gametes are either not formed or that sexual fusion is impossible.

The part that trace elements play in the development of the ovary and in ovule production is not known. Work such as Reed's on seed production, Scharrer's [251] showing the beneficial influence of copper in hastening flowering in oats, and clinical observations on deficient plants which show lack of either ovary development or ovule formation, make it clear that the micro nutrients are involved in ovum production, although their exact part is quite unknown.

Animal reproduction

In animals more information about the influence of trace elements on reproduction is available than in plants. Iodine, manganese, and copper play a very important role, and their imbalances result in well-known reproductive failures. Zinc is also of importance, although its reproductive role is not so well known.

Iodine in mammalian reproduction

The widespread deficiency of iodine in farm animals gives rise to much infertility. If young are born they are often dead at birth, or weak and hairless, as the piglet in Fig. 47. The parents may be normal, but their progeny have a very low iodine content in their thyroid glands [12]. Lack of iodine can cause goitre, with its 'big neck', but the presence of this swollen thyroid is not necessary for impaired reproductive ability. The link between normal thyroid functioning, where iodine is needed to form the iodine-containing hormone thyroxine ($3:5:3^1:5^1$ -tetraiodothyronine) and normal sexual development is very close. Thus, in conditions such as cretinism in human beings, which is due to a disturbed iodine metabolism, the unfortunate sufferer is usually sterile, has delayed maturation of the genitaliae, and invariably fails to develop normal sexual vigour [306] (cf. Fig. 54, page 127). When thyroidectomy, i.e. removal of the thyroid gland, is performed on young birds and mammals the animal stays in an infantile state for a long time, and gonads and secondary characters are developed very much later than is normally the case.

The administration of the iodine-containing hormone thyroxine to bulls, boars, and rams that have shown little sexual desire results in a marked improvement in libido. Thyroidectomy, on the other hand, results in loss of libido, and in cows a complete lack of oestrus, although neither spermatogenesis nor ovulation are impaired [14]. In the field, cows on iodine-fertilized pastures had stronger heat and were more fertile, conceiving in fewer copulations per conception, than those on normal pastures. Their milk yields were also higher [252].

Apart from its part in influencing oestrus, iodine is also important



FIG. 47

GOITRE IN ANIMALS [12]

Hairlessness and myxoedema in piglets suffering from severe goitre

This piglet shows the extreme hairlessness and myxoedema of acute goitre in piglets. The parent sows may be apparently healthy, but the iodine content of the piglet's thyroid gland is extremely low.

This condition is very common in certain parts of the world, and is of great economic importance.

in the intra-uterine development of the mammalian embryo. In iodine-deficient areas abortions and still births are common, and retention of the placenta is frequent. The gestation period may also be prolonged, and births may be difficult. In birds iodine deficiency can result in longer incubation periods, with higher percentages of dead embryos (cf. Table 20, page 77).

In humans the greatest iodine requirements appear to be linked to the phase of sexual development in females. The increased demands for thyroidal function which are known to be associated with menstruation, pregnancy, and lactation, may well explain the observed high incidence of goitre and thyroid disease in women [18]. Colloidal

goitre frequently first exhibits itself at puberty, while hyperthyroidism is often developed at menopause. The ovaries contain a high level of iodine, but this is lower before puberty and after menopause.

In human pregnancies there is an appreciable rise in the protein-bound iodine (PBI) of the blood. The significance of this is unknown, and this phenomenon is not observed in other mammals, such as cows, but if this rise does not take place, the pregnancy is apt to end in an abortion. Yet this rise in PBI is not linked with any obvious clinical condition.

Certainly hypothyroidism of such a slight extent that signs of goitre are hardly detectable can have a marked influence on the abortion rate [152]. As is shown by the figures in Table 25, the

TABLE 25

Iodine as a factor in human abortion. From KING AND HERRING [152]

CLINICAL CONDITION	NUMBER OF WOMEN	ABORTIONS	
		PREVIOUS*	PRESENT
Hyperthyroid	17	3	1
Normal	72	4	1
Hypothyroid	61	13	8

The rate of abortion in hypothyroid women is much greater than in normal ones. The degree of hypothyroidism need only be slight, and goitre may not be readily observable, in order seriously to affect reproduction.

* Abortions prior to this investigation.

abortion rate is markedly increased in both hypo and hyper-thyroidism.

The undoubted influence of iodine in the terminal stages of human pregnancy is clearly shown in Table 26. Women who received iodine

TABLE 26

The influence of low levels of iodine on the incidence of stillbirths. From KEMP [149]

SERIES	IODINE ADMINISTERED			NO IODINE ADMINISTERED		
	TOTAL DELIV- ERIES	IDIO- PATHIC STILL- BIRTHS	PER- CENTAGE INCI- DENCE	TOTAL DELIV- ERIES	IDIO- PATHIC STILL- BIRTHS	PER- CENTAGE INCI- DENCE
1. 1925-29	2,252	2	0.089	14,532	82	0.546
2. 1930-32	742	1	0.134	4,071	49	1.203
TOTAL	2,994	3	0.100	18,603	131	0.700

Unexplained or idiopathic stillbirths in white women living in Vancouver were investigated. Women who received therapeutic iodine in their last three months of pregnancy had a much lower incidence than did untreated women. Local Indians in the area, who live on fish and other iodine-rich foods, have virtually no unexplained stillbirths, or goitre.

therapy in the last three months of pregnancy had a low incidence of unexplained or 'idiopathic' stillbirths, compared to those who had no treatment. This condition was virtually unknown among nearby Canadian Indians living mainly on salmon and other iodine-rich foods [149]. These observations must be borne in mind when goitre and its causes are discussed later.

Manganese and reproduction

Manganese has a marked influence upon both male and female animals. In male rabbits and rats deficiency leads to a lack of libido, but in contrast to iodine deficiency, it is also accompanied by a marked testicular degeneration, with lack of spermatozoa, and hence male sterility. The precise function of manganese in spermatogenesis is not known.

In female rats the deficiency leads to the oestrus cycle being upset and inadequate development and functioning of the mammary glands. After prolonged deficiency, i.e. over ten months, the oestrus cycle was extended considerably, and females remained in the post-oestrus or anoestrus stage for weeks. Mating did not take place in such animals [278].

Shils and McCollum [278] distinguish three degrees of manganese deficiency in female rats, depending on the severity of the deficiency. In the first stage viable young are produced, but these suffer from ataxia or inco-ordination of movement and sometimes paralysis. The second stage is when non-viable young are born, who die shortly after birth. In the third and most extreme stage there is little or no oestrus, a marked delay in the opening of the vaginal orifice, and the animal will not mate; hence complete sterility. Yet in spite of these very pronounced symptoms, there are no marked alterations of the ovaries as a result of the deficiencies, although the manganese level is very low and does affect the development of the embryo in the uterus.

While the above investigators claim that manganese deficiency does not affect mother-love or willingness to suckle, Grashuis [116] found otherwise. He claimed that oestrus was normal, but mother-love and milk production were impaired by the deficiency. As conditions were not comparable, and other factors are involved in reproduction, this contradiction may be more apparent than real.

In cattle manganese levels are certainly an index of fertility, and deficient heifers are slower in showing oestrus and in conceiving than normal ones, while the calves they produce tend to be weak [116]. As the ovary reflects the manganese status of the feed very clearly, the ovary content of manganese is low in these animals. Thus, cows with adequate manganese had a content of 2.0 p.p.m. manganese in their ovaries, while deficient ones had 0.85 p.p.m. [32].

Manganese content of cattle hair can be used as a gauge of the

manganese status of the animal. In Hanover animals with a hair content of less or more than 10–20 p.p.m. manganese had decreased fertility, as the figures in Table 27 show [188].

TABLE 27

The relationship between the fertility of cows and the manganese content of their hair. From MEYER AND ENGELBERTZ [188]

MANGANESE CONTENT OF HAIR, P.P.M.	INSEMINA- TION INDEX*	NUMBER OF INSEMINA- TIONS	NUMBER OF COWS IN GESTATION	GESTATION PER- CENTAGE
Less than 10	1.71	103	53	51.6
10–20	1.56	232	144	62.2
More than 20	2.00	108	50	46.3

* The number of inseminations for one live birth. 351 cows were studied, with an average hair content of 15.8 p.p.m. manganese and a range of 3.9–49.9 p.p.m. Mn.

The administration of oral manganese resulted in a rapid rise in the manganese content of the hair. When it was stopped the manganese level of the hair dropped to its original level in eight weeks.

A manganese content of more than 20 p.p.m. or less than 10 p.p.m. in the hair indicates a decrease in fertility.

In swine insufficiency of manganese leads to disturbances of reproductive cycles, but ovulation apparently continues more or less normally [225].

As far as humans are concerned, nothing is known about the influence of this element. It is doubtful if deficiencies of this would occur in most communities, but in some under-developed areas deficiencies could exist. For instance, the unexplained infertility in parts of Northern Nigeria described by Nicol [212] may involve some mineral deficiencies. Certainly animal and plant deficiencies are widespread in Nigeria and in Africa in general.

Copper and reproduction

Copper is another element that plays an important part in reproduction. The fact that low levels of copper are associated with poor growth and retarded milk production (Fig. 24, page 60) constitute serious agronomic problems. But copper deficiency also results in impaired fertility in cattle [117, 310]. In areas such as Britain [2], where copper deficiency may be molybdenum induced, the decrease in fertility may also be due to toxic influences of this element damaging tissue. The reproductive difficulties experienced, particularly by heifers, may not be due exclusively to lack of copper.

However, Dutt and Mills [93] investigated the influence of copper deficiency on the reproduction of rats in some detail. They were able to feed the animals on a purified diet and to control the copper

content of the animal's food. In hooded Lester rats, in which only females were fed low copper diets, the oestrus cycle of the deficient animals was quite regular, and all animals conceived with no difficulty. But rats with less than 2 p.p.m. copper in their diet did not litter. At low levels of copper only 3 rats in 18 produced young, but these were normal. In the majority the animals conceived, gained weight, and aborted, which then resulted in a very rapid loss of weight. It was observed that the does had a cyclic increase in weight, and then a sudden drop, showing the regular conceptions and abortions taking place. Dissection showed that the foetus was resorbed immediately after death. It appears as if the copper deficiency restricts the rapidly growing embryonic tissues much more severely than the more mature maternal tissues.

The picture in ruminants is not so clear. However, it appears that the oestrus cycle is upset in heifers, who may abort and skip a few heats [79]. But the general picture is not so well understood as in the case of manganese deficiency.

Zinc in mammalian reproduction

Zinc is an element that is present in fairly large quantities in mammals, being about half the concentration of iron and considerably more than that of such elements as copper. Yet its function in animals and man is not at all clear.

In reproduction zinc is particularly associated with the testicles and sperm, and in experimental rats a deficiency leads to irreversible atrophy of the testes, and hence sterility in the male [190]. If zinc-deficient rats are given gonadotrophin it brings about an increase in the size of the prostates, seminal vesicles, and coagulating glands, while a lesser development of testes and epididymus ensues. Thus, it appears that zinc deficiency probably influences male reproduction by depressing gonadotrophin production [97]. The zinc content of viable sperm, especially human sperm, is very high, and this element is of importance as a constituent of these cells [183].

In the light of these facts, and similar findings not cited, it is difficult to agree with Underwood's [306] opinion that the essential nature of zinc in mammalian reproduction has not been established. Certainly one must agree that *naturally occurring* zinc deficiencies, causing reproductive upsets, are very rare.

Trace elements in reproduction

The general picture that emerges from an investigation of the part played by micro nutrients in reproduction is that they are important in many phases of this process, and that while the exact details of the chemical mechanisms in which they are involved are by no means clear, their essential nature is quite obvious. Nor must it be over-

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looked that in reproduction a great deal of energy is required, as the rate of respiration of structures such as sperms is very intense. This implies a need for elements such as iron, copper, and manganese, as was shown in Chapter II, in order to allow normal energy transfers to take place. The link between trace elements and normal reproduction is obviously a very strong one.

VIII | Health and disease as a function of trace element levels

The fact that disturbed trace-element nutrition can result in appreciable anatomical abnormalities characteristic of these states has already been discussed in Chapter VI. But other differences between healthy and diseased organisms, particularly biochemical ones, may be equally striking, and of great importance, as this chapter will show.

It is very difficult to find a concept that really describes what health is. It is far more than an absence of disease. On the other hand, slight malfunctioning of certain organs is so normal that the slight adjustments involved cannot be regarded as unhealthy symptoms.

Nor can health be very readily expressed in a few simple clinical tests, and certainly its presence or absence is not easily determined by measurement of a few trace-element concentrations. For in conditions such as essential pulmonary haemosiderosis, iron is deposited in large quantities in the lungs, yet an acute anaemia exists at the same time. Account must also be taken of the varied requirements of different organisms and their ability to adapt themselves to a variety of conditions. Accumulator plants and animals can absorb very high levels of elements that would be toxic to most forms of life. Again, antagonisms among elements lead to an altered mineral requirement. These factors have been mentioned before, and will be discussed in the next chapters, but they must be borne in mind when health and disease are considered.

It is not always appreciated that in plants it is not easy to determine health. Vigorous growth is only one criterion, and it can exist in plants that are susceptible to infections, or cold, or drought. Yet this susceptibility must also be regarded as an absence of complete health, because plants are, in fact, exposed to these influences, and healthy ones can resist them to a considerable extent. Still some indications of well-being, such as chlorosis, do exist in plants.

Chlorosis

The green pigment chlorophyll is present in the leaves of plants, and is responsible for their green colour. In most higher plants the leaves of healthy individuals are a uniform fairly dark-green colour. How-

ever, in mineral deficiencies, and to some extent in excesses, this uniform pigmentation is upset, and the leaves may be pale green with dark-green veins, or only the inner part of the leaf may be pigmented, etc. A large number of pigmentation patterns are known. This uneven pigmentation is known as *chlorosis*. A perusal of books on plant deficiencies will show the characteristic chlorotic patterns associated with various imbalances [319].

As chlorophyll is the photosynthetic pigment in plants which enables light energy to be used for the synthesis of organic molecules, it is clear that a marked reduction in chlorophyll content, or distribution in the leaf, must have an influence on photosynthesis.

The main pigments in the chlorophyll complex are chlorophyll a and chlorophyll b, which are complex porphyrins. Chlorophylls are similar to such substances as haemoglobins, except that they contain magnesium instead of iron in their molecules.* Certainly, deficiencies of manganese, iron, and zinc result in reducing chlorophyll synthesis very appreciably, although what stage of the synthesis is blocked by absence of these elements is not clear. It is some process before the introduction of magnesium into the molecule, which only takes place when the synthesis of the molecule is nearly completed [223].

A very analogous state of affairs is found in haemoglobin synthesis, where cobalt, copper, manganese are known to be involved in the synthesis, as well as iron, which is part of the molecule. It is probable that vanadium may also play a part in this very complex synthesis [304], but not all the stages are known with certainty. But lack of the above elements can result in anaemias.

Severe chlorosis leads to decreased photosynthesis, and hence to impaired growth. Elements like *chlorine* also play a part in this process. Chlorine has been shown to be essential for the photochemical processes involved in photosynthesis [22]. Boron, on the other hand, is not directly involved in either the formation of chlorophyll or in the synthetic process itself, but influences photosynthesis by regulating the rate of sugar translocation from the cells involved [111].

Iron in mammals

The varying need of organisms for different mineral nutrients can be well exemplified by reference to iron, and to a lesser extent copper, in mammals and particularly in humans. The differing requirements at different stages in development can be clearly seen, and the manner in which the very widespread simple iron-deficiency anaemia results in loss of health and vigour can be understood.

* There are also other differences, especially in the lateral chains attached to these molecules.

In normal healthy humans there is a considerable store of iron, especially in the liver, which is the main iron storage organ, and in the blood, which has a large quantity in the red iron-containing respiratory pigment, haemoglobin. The storage iron is present as the protein ferritin (20 per cent iron), and as haemosiderin, a colloidal ferric hydroxide phosphate which may contain up to 35 per cent iron.

Human infants are born with a very high level of haemoglobin in their blood. This rapidly breaks down, and the iron is used to meet the normal nutritional requirements, and during the first year of life there is a marked drop in haemoglobin levels in the blood, from about 22 g./100 ml. blood to around 12 g./100 ml. blood. Then there is a gradual increase again.

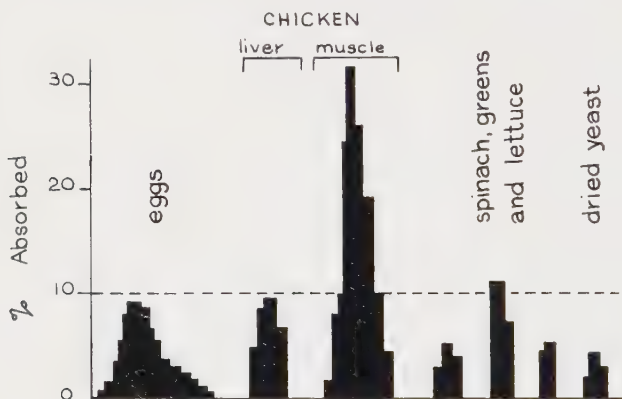


FIG. 48

SEIBOLD [276]

The percentage of iron absorbed from food

Humans seldom absorb more than 10 per cent of the iron in food. Certain conditions, such as gastroenteritis, lack of acid in the stomach, or removal of part of the stomach, may decrease this amount even more, and so result in iron-deficiency anaemia.

Due to the relatively large liver of the human infant with its high content of iron, and its high level of haemoglobin in the blood, anaemias in infancy are not conditions that might be expected, even though milk is a very poor source of iron. But hypochromic anaemia (a deficiency condition) is not uncommon. It is not associated with depletion of the iron reserves, but with an inability to use them, and this condition responds well to iron therapy.

Naturally occurring iron-deficiency anaemia in animals is almost unknown except in stall-fed pigs, where animals reared on concrete-floored pens may show it. Piglets are unusual in that they contain very little iron, and have no appreciable liver reserves or high levels of haemoglobin. As piglets grow very rapidly (twice as fast as calves),

the low iron content of milk is not sufficient for normal development, and they develop 'thumps', an anaemia associated with very laboured breathing, which is frequently lethal. On open pastures these animals would meet their iron requirements by rooting around, but in pens their iron requirements frequently must be met by such procedures as injections of iron salts [306].

Although iron is present in many foods, it is only partially available. Many problems arise from this difficulty in absorbing this element, for simply eating iron-rich foods does not result in increased absorption by the gastro-intestinal tract. The histogram in Fig. 48 shows what a small percentage of the total iron is absorbed by normal healthy people from various foods.

Differences in iron requirements between the sexes

Iron is an interesting example of an element that is required in appreciably larger quantities by women than by men. Up to the age

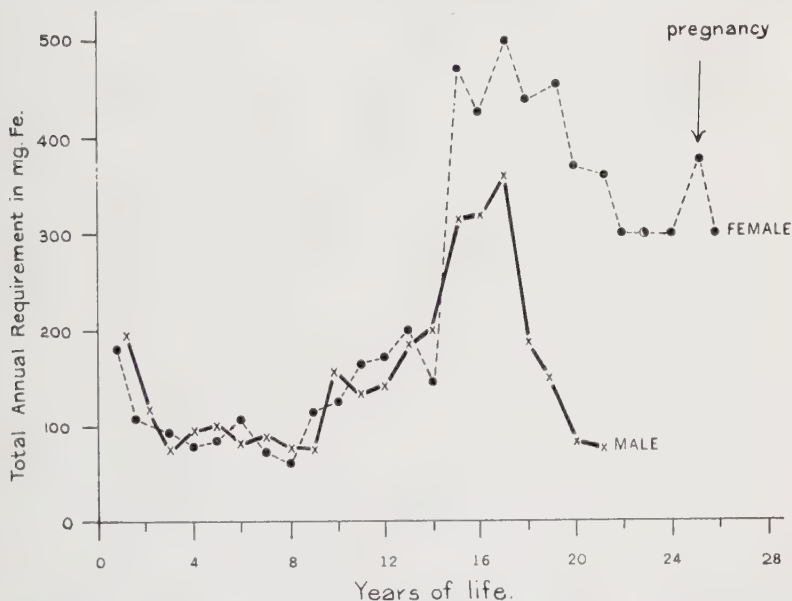


FIG. 49

AFTER UNDERWOOD [306]

Differences in iron requirement between men and women

The iron requirements of children are similar, irrespective of sex, until puberty is reached. Then those of the female rise to a much higher value than for the male, and never drop to a similar level. Pregnancy enhances the iron requirement temporarily. Even in later life, after the menopause, the iron requirements of the female exceed those of the male.

of puberty the iron requirements of both sexes are almost identical, but with the onset of sexual maturity in women, menstruation, gestation, and lactation all make heavy demands on the iron reserves present. During their fertile years women lose on an average of 200 mg. iron per annum through their menstrual flow. In pregnancy, although loss of iron due to menstruation ceases, the growth of the foetus and its associated structures are a heavy drain on iron, and parturition involves a loss of about 350-450 mg. iron. It is not surprising that nutritional anaemias are more frequent in women than in men, and that mild anaemias are commonly found in pregnancy. Although iron is secreted in the milk, it seems that lactation has never been found to induce simple iron-deficiency anaemia.

These losses of iron obviously result in a greater iron requirement in women than in men, as can be clearly seen in Fig. 49. It is more difficult to understand why, after the menopause when these losses of blood, etc., are no longer incurred, the requirements of iron in women should still remain higher than that of men.

Copper in mammals

Copper is normally present in much lower concentrations than iron, and in man is present at about $\frac{1}{10}$ the concentration of non-haemoglobin iron. Just as with iron, new-born infants have a much higher level of copper than adults do. In the new-born human infant the copper content is around 4.7 p.p.m. copper, as opposed to the 1.7 p.p.m. level in adults. Although the liver is the main storage organ for copper, appreciable quantities are present in muscles and bones. It is also present in blood, where the fluctuations of copper are of real interest.

Human milk (and other milks) are poor sources of copper, and the copper content decreases with length of lactation. Colostrum is richer than ordinary milk in this element. The fact that the liver of infants has a very high copper content of around 230 p.p.m. dry matter, which is 5-10 times the normal adult level, led to the opinion that these large copper reserves were present to meet the needs of the infant until it was weaned. Authorities such as Marston [180] claimed that mammalian young must have liver reserves of copper because of their milk diet. Unfortunately, it has often been overlooked that while liver copper decreases during breast feeding, the rapid growth of the child results in an *increase in total copper* in the body, and that the infant obtains more copper from milk in a few weeks than it had in its liver at birth. This does not imply that liver reserves are not important, but only that they can be over-emphasised.

The fact that the mineral elements can be redistributed in pregnancy was already shown when the marked increase in protein-bound iodine of human blood serum was mentioned (page 105). As a rule,

there is also a drop in the serum-iron concentration during pregnancy, which is something that might be expected. In pregnancy there is a marked *increase* in serum copper, and at full term the copper level may be two or three times the normal concentration. This rise in serum copper would be a very simple and useful way of diagnosing pregnancy, were it not for the fact that many diseases and infections, including the common cold, also result in this increase [306, 314].

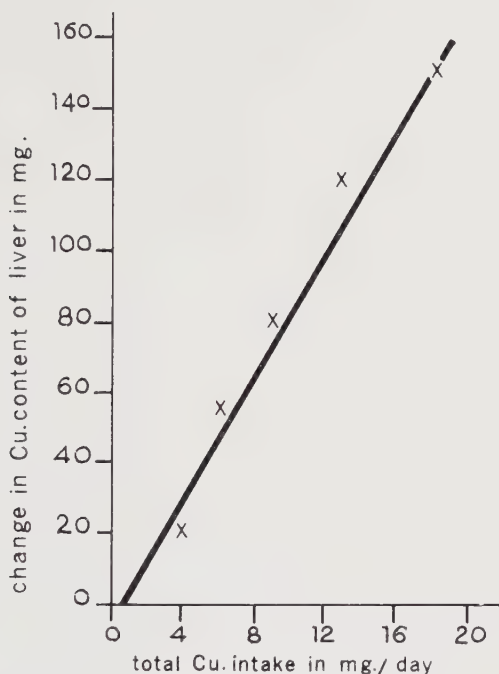


FIG. 50

AFTER DICK [87]

Relationship between copper intake and liver copper levels in sheep

The concentration of copper in the liver is a very good reflection of the level of copper in the diet of sheep.

In cattle, where copper deficiencies cause extensive practical problems, it is desirable to assess the copper status of the animals in order to know how to treat them. It has been claimed that manganese status and its influence on reproduction can be determined by measuring the manganese content of the hair (Table 27). Unfortunately, blood copper is not a reliable index, but liver biopsies can be undertaken in living animals [308]. As the liver is the main store for copper, and its copper levels are not subject to sudden and rapid fluctuations, this is a very valuable technique. In sheep the liver is also a good

index of copper status, and Fig. 50 shows the linear relationship between liver copper and copper intake [87].

Anaemia and haemopoiesis

Both copper and iron play an important part in anaemias. Simple deficiency of iron results, among other things, in insufficient iron being present for the formation of haemoglobin molecules. But iron-deficiency anaemia is more complex than this.

Both iron and copper are directly involved in haemoglobin formation. Iron is directly involved in both haeme and globulin synthesis, while copper is primarily active in the formation of reticulocytes [181]. However, Gallagher [105] shows that copper is involved in haeme α synthesis in rats, while Howell and Davison [140] show that it is directly involved in haeme synthesis in lambs. The latter also claim that cytochrome oxidase contains copper.

It is of interest that iron is not incorporated into all the haeme-containing enzymes with equal ease. Even in severe iron deficiency there is no reduction in catalase activity, and its synthesis is apparently more successful than that of haemoglobin, when there is competition for iron [34]. Catalase activity was not decreased by copper deficiency in rats either, but within one month of being placed on a deficient diet the liver cytochrome oxidase activity had dropped by 76 per cent.

Anaemia was a relatively late symptom of copper deficiency, and it took ten weeks on a deficient diet before haemoglobin values started to drop [105]. As cytochrome oxidase is the terminal oxidase in rats, copper deficiency led to depletion of this enzyme, and this is apparently the immediate cause of death. That this is the case seems to be borne out by the fact that forcing the animals to move, and exciting them, hastens death [104]. In swine, copper deficiency results in a marked decrease in cytochrome oxidase activity in the heart [121], while lack of iron does not do this to nearly the same extent, cf. Table 28, page 117. Yet *copper deficiency anaemia is morphologically indistinguishable from iron deficiency anaemia* [60]. Cartwright and his co-workers [60] are of the opinion that lack of copper in some way hampers the utilization of iron in the organism, and this is one of the reasons why iron injections or oral iron cannot cure this anaemia. In pigs the copper deficiency anaemia appears to be due to two factors. First, the very impaired production of red blood cells in the bone marrows, and, secondly, the very appreciable reduction of the erythrocyte life span, compared to normal ones [61].

However, more trace elements than copper and iron are involved in blood formation. Cobalt, which is a constituent of vitamin B₁₂, also plays an important role. In Finland people infected by the fish tapeworm *Diphyllobothrium latum* eventually suffer from anaemia caused

by lack of vitamin B₁₂. These carriers absorb less than 55 per cent of the normal amount of this vitamin. If the worms are removed, the anaemia is readily cured [214].

TABLE 28

Some haeme-containing substances expressed as a percentage of their normal value, in copper- and iron-deficient swine. From CARTWRIGHT et al. [61]

ENZYME	COPPER DEFICIENCY	IRON DEFICIENCY
Cytochrome C (heart)	124	46
Cytochrome oxidase (heart)	10	65
Catalase (kidney)	121	104
Myoglobin (muscle)	99	25

The turnover rate of iron in both blood plasma and red cells is greatly enhanced in copper-deficient pigs. If the mobilization of iron were seriously impaired by copper deficiency, then it might be expected that the activity of the haeme-containing enzymes would be reduced. As can be seen, this is not true, although the cytochrome oxidase activity of the heart is greatly reduced, the other haeme-containing compounds are not, even in severely copper-deficient animals.

The beneficial influence of vanadium upon haemopoiesis is stressed by Trummert and Boehm [304], and it is of real value in the treatment of anaemias. In rabbits its application doubles reticulosis. Certainly, mixtures of vanadium, cobalt, and copper have a very stimulating effect upon red blood-cell formation in blood [304].

Clearly, anaemias can have many and varied causes.

Goitre and upset iodine metabolism

Goitre is a disturbance of the thyroid which results in the enlargement of this gland, to give the typical 'big neck' condition, cf. Chapter VI. This enlargement results in inadequate production of the iodine-containing hormone, thyroxine, which is the main hormone controlling metabolic rate. Because of this, goitre is clearly a more complicated condition than an unsightly swelling of the neck. Goitre is very widespread, and in many cases a simple iodine deficiency is involved, so that prophylactic supplementation of the diet with iodine may prevent this condition by supplying adequate amounts of iodine for thyroxine manufacture by the thyroid gland (cf. Table 24, page 98). But iodine does not cure all goitres, so the etiology of goitre, although it is undoubtedly affected by iodine supplies, must be more complex.

Goitre may be absent in areas low in iodine and still persist in areas with adequate iodine, or where iodine supplementation is practised. Goitrogenic factors, which promote goitre by increasing the iodine requirements of the body by antagonising the iodine and making it unavailable to the thyroid, are very important in some

areas. The thiocyanates in the brassicas (cabbage family), thiourea, excess calcium, and sulphonamides all act as goitrogenic factors in the diet, while high levels of the halogens, chlorine, bromine, or fluorine may induce goitre by stimulating the excretion of iodine from the body.

In Finland [219] cattle were studied in areas where iodine prophylaxis had been practised for thirty years, and in spite of the plentiful supplies of iodine, the animals' thyroids were 60–90 per cent heavier than those of non-goitrous areas. The iodine *concentration* of these glands was 30–40 per cent lower than that of the non-goiterous beasts, although the *total* iodine content was the same in both groups. Similarly, iodine treatment was unable to stop the development in humans.

Heavy doses of iodine have been reported to cause goitre, but how this happens is not clear. Burrows *et al.* [55] are of the opinion that this iodine-induced goitre is more prevalent than is generally recognized, and Wilflingseder [329] has experimentally induced goitre in guinea pigs within four weeks by giving them twenty times the normal daily dose of iodine.

Adequate supplies of copper appear to be necessary for the utilization of iodine, as the first stage in the formation of thyroxine from iodine is the non-enzymatic catalysis of iodine and tyrosine to form monoiodotyrosine, although both iron and hydrogen peroxide appear to be able to promote this reaction [99, 326]. Certainly there is a clearly established interaction between thyroxine and copper, as the level of the hormone appears to regulate the level of the copper in the blood [205, 242].

Cobalt is an element that also plays a part in thyroid metabolism. Very small quantities of vitamin B₁₂ prevent the toxic effects of excess thyroxine (thyrotoxicosis) in rats [294] and man [113]. It is possible that the first signs of thyrotoxicosis are, in fact, vitamin B₁₂ deficiency. In spite of its beneficial effects in cases of excess thyroxine, cobalt may in certain circumstances interfere with thyroid function and induce goitres. When cobalt therapy has stopped the goitres recess. Only a limited number of people appear to be prone to this form of goitre [10].

The fact that goitre is common in pregnant women has been discussed in the last chapter, as was the fact that lack of thyroid activity reduces the reproductive ability of man and animals. Lack of vitamin B₁₂ also leads to reproductive failure. These may be related. Rats with their thyroids removed did not become pregnant unless vitamin B₁₂ was supplied, and for normal fertility dried thyroid had to be supplied as well [67]. In rats lacking a thyroid oral absorption of vitamin B₁₂ was much reduced, but if thyroid hormones are given to these animals the absorption returns to normal [215].

This discussion shows to some extent the complexity and the variety of some of the causes of goitre. It is by no means complete, and does not include such items as the reports of goitre whose cause is unknown, as for instance, the apparently rapid rise in goitre incidence

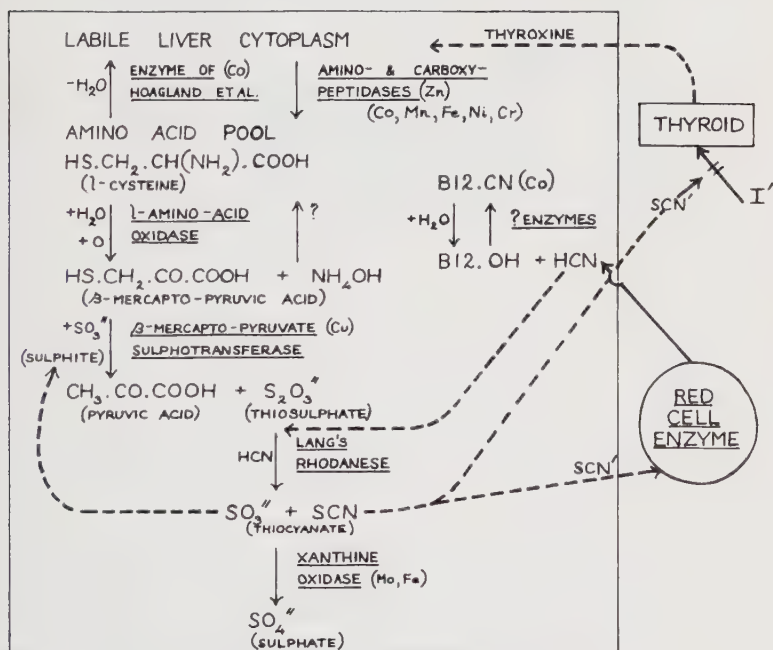
TABLE 29

Surprising increase in the thyroid enlargement in women attending the antenatal clinic in Dorset, England. From LISNEY [169]

	1945	1946	1947	1948	1949
Total women examined	283	332	304	318	178
Percentage with enlarged thyroid	6.36	3.9	7.23	5.03	15.73

These figures must be accepted with great caution, as it is not clear how representative they are, and the total number of women examined in 1949 was small. There is no known physiological reason why women should be goitre-prone *after* parturition.

reported in an ante-natal clinic in Dorset, England [169], cf. Table 29. Owen has very successfully integrated a large number of these



METALS AND LIVER ENZYMES

FIG. 51

OWEN [217]

Suggested role of trace elements in the self-limiting action of the thyroid gland

observations into a scheme which shows how a number of these interactions enable the thyroid gland to function as a self-regulating mechanism [217]. A slightly enlarged scheme is depicted in Fig. 51, and gives some idea of how complicated the interactions actually are in biological systems.

Cancer and trace elements

Trace elements appear to play some part in cancer. It is quite certain that in man the trace-element distribution is disturbed as a conse-

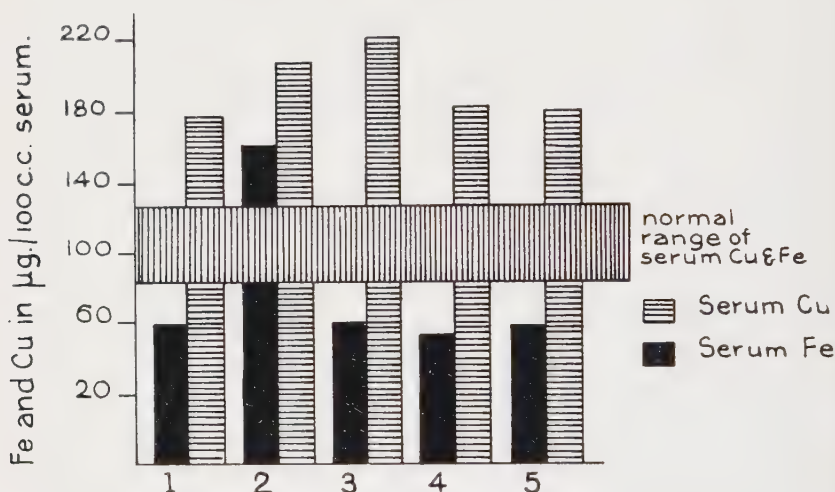


FIG. 52

AFTER KEIDERLING AND SCHARPF [147]

Fluctuations in serum copper and iron in a number of cancers

1. Chronic leukemias (64 cases).
2. Acute leukemias (28 cases).
3. Lymphogranulomatoses (17 cases).
4. Bronchial carcinoma (19 cases).
5. Carcinoma of the stomach and intestines (23 cases).

It is clear that in cancers the blood-serum level of copper is appreciably enhanced, while that of iron (except in acute leukaemias) is markedly decreased.

quence of this disease. The iron and copper content of the blood serum are always disturbed, and the data in Fig. 52 show that cancer elevates the copper level of the serum in all cases, and that the iron content is well below average, except in acute leukaemia [233]. Keiderling and Scharpf [147] had shown substantially the same results previously, as had Pirrie [224].

Voisin [314], in his study on cancer and soil, lays great stress on the important role that copper may play in cancer. It is interesting

that Kiederling, working from clinical data, came to a similar conclusion quite independently [147].

Halme [123] has demonstrated experimentally the important part played by high levels of zinc in bringing about mice carcinomas. He suggests that zinc accumulation may be an important factor in man, and points out that in his native Finland 35 per cent of the drinking waters contain high levels of zinc. Certainly, upsets in the zinc metabolism have been observed in other cancers. The prostate gland normally is the tissue with the highest level of zinc in the body. But cancer of this gland reduced the zinc level from around 850 $\mu\text{g./g.}$ dry weight in healthy ones to 190 $\mu\text{g./g.}$ dry weight in cancerous glands [182].

On the other hand, some trace elements have on occasion been shown to exert an inhibiting influence on cancer development. Thus, manganese malate inhibits new growths of tumours in rats and mice. Apparently it alters the metabolism of tumours, and tends to promote the normal aerobic respiration of cells, in place of the pathological anaerobic respiration of cancer cells [25]. Other workers have shown that copper compounds can have an inhibiting effect on tumour formation [296, 139]. Bergel in his book on cancer [33] points to the beneficial therapeutic action of vanadium in certain kinds of cancer. Again, this shows the biological importance of this element, even if it does not prove its essential nature.

Voisin [314] lays great stress on the fact that in many parts of the world cancer incidence is not uniform, and in fact appears to be a condition linked to the environment. Thus, the maps in Fig. 53 show the uneven distribution of stomach cancer in North Wales, and how there is a correlation between the soil organic matter level and cancer [167]. Further investigations have revealed that not only organic matter but zinc, cobalt, and chromium levels in the soil are positively related to stomach cancer [287].

This apparently soil-linked cancer is also found in parts of Africa. There is a 'Lymphoma Belt' stretching from east to west across equatorial Africa [53], in which this lethal cancer is common in children. No real correlation between this condition and the environment have yet been established.

However, in the Transkei, South Africa, oesophageal cancer, a new disease in this under-developed area, is increasing rapidly among the black people living there. Again this disease is not uniformly distributed, and 89 per cent of the cancerous regions are found on poor soils derived from Beaufort sedimentary rocks. On the other hand, 64 per cent of the completely cancer-free sites were on rich soils derived from igneous dolerite. It looks as if the poor soils produce food that is progressively less nutritious as a result of soil impoverishment; this may play a part in carcinogenesis [54].

As was shown, endemic goitre is a condition that is linked to an environment. It is thus interesting to see that some Swiss workers claim that there is much clinical evidence to show that there is a relationship between endemic goitre, thyrotoxicosis, and carcinoma of the thyroid, and what is more, iodine supplementation has been claimed to lower both the incidence of endemic goitre and of thyroid carcinoma [328].

At our present state of knowledge about cancer it is difficult to

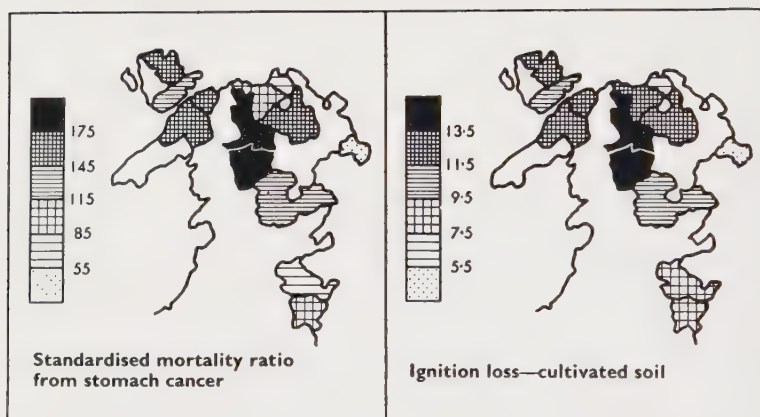


FIG. 53

FROM LEGON [167]

The uneven distribution of stomach cancer in North Wales compared with the distribution of soils with high organic matter content

The maps above show the non-uniform distribution of stomach cancer in Wales. It is high in regions which have a high organic matter (ignition loss) content in the soil, and low in other areas. There is a correlation between cancer and high organic matter in the soil. This does not mean that the two factors are necessarily linked, or that they influence each other; it does mean that they may be, and that further investigations should be conducted.

In this case the high soil organic matter may induce a copper deficiency in the soil. Voisin [314] discusses the implications of this and similar conditions in some detail.

see all the implications of this information, but it leaves little doubt that the trace elements do play a part in the development of, as well as the resistance to, this lethal disease.

Environmentally linked diseases

Goitre and cancer are not the only environmentally linked conditions. Dental caries is often linked to the low level of fluorine in the local water, while excess results in debilitating fluorosis (Chapter VI). The same is generally true of the other deficiency diseases in animals,

while toxicities, such as selenium poisoning or alkali disease (Fig. 7, page 37), certainly are.

In Russia a number of these conditions have been observed [157]. In North-West Kazakhstan high levels of boron in the soil produce enteritis in 3.5 per cent of the sheep and some of the children of this area; in parts of the Armenian mountains molybdenum-rich regions may have up to 30 per cent of the adult population suffering from gout (podagra); nickel-rich soils result in the accumulation of this element in the corneas of lambs and calves, with consequent eye disease; in the Amur regions animals grazing on soils low in calcium but high in strontium and barium have very deformed joints.

However, the influence of the environment may be very complex, and Voisin [314], in his study of cancer, has shown the kind of factors in soil-plant-human relationships that may be involved in these soil-linked conditions.

Cause and effect relationships

For the sake of clarity, it is necessary to point out and stress that even in the most carefully conducted investigations *correlations, even if they are statistically very significant, do not prove any cause and effect relationship*. The fact that a disease and some factor very frequently occur together does not mean that the factor causes the disease. This very important point is frequently overlooked, or completely ignored, and results in some very unscientific conclusions. Street lights always burn at night, but not during the day, and there is a very strong correlation between darkness and the lamps burning, but this does not mean that darkness is the factor which switches on the lights. Many other factors are involved. So it is with the correlation between low available soil copper and high zinc levels and the high incidence of certain kinds of cancer. The elements possibly play some indirect part in causing the cancer, but this must be elucidated by further research work.

Atherosclerosis and cholesterol

Atherosclerosis is a very important disease which is extremely widespread in more developed and prosperous countries. This condition is associated with high levels of blood-serum *cholesterol*, and the medical treatment is concerned primarily with trying to reduce the serum cholesterol levels.

The fact that vanadium can inhibit cholesterol synthesis is thus of great practical interest [20]. Curran and Costello [78], demonstrated experimentally that non-toxic quantities of vanadyl sulphate (0.05 per cent) in the diet inhibited the cholesterol synthesis in the liver of rabbits. This reduced the excess aortic cholesterol and clearly demonstrated the anti-atherosclerotic activities, for it is the beading

of fatty compounds in the main arteries and the heart that is so characteristic of this disease. Autopsy showed that, even in rabbits which had been fed on cholesterol, the formation of these structures was hindered by vanadium. Table 30 shows the comparison between vanadium-fed rabbits and those lacking this element.

TABLE 30

The cholesterol levels of sera and tissues from cholesterol pre-fed rabbits before and after six weeks on vanadium-supplemented diets. From

CURRAN AND COSTELLO [78]

		MEAN CHOLESTEROL CONTENT, IN MG. %
Initial serum level	V diet*	937 $\pm$ 86.4
	Control†	955 $\pm$ 79.9
Serum after 6 weeks vanadium supplementation	V diet*	36.7 $\pm$ 8.4
	Control†	65.2 $\pm$ 17.7
Level in aorta after 6 weeks supplementation	V diet*	13.8 $\pm$ 2.69
	Control†	26.6 $\pm$ 2.97
Level in liver after 6 weeks vanadium supplementation	V diet*	7.99 $\pm$ 2.4
	Control†	8.24 $\pm$ 1.5

* V diet contained 0.05 per cent $\text{VOSO}_4 \cdot 2\text{H}_2\text{O}$.

† Control diet contained no vanadium.

The initial very high levels of serum cholesterol were due to the pre-fed cholesterol, and there was no significant difference between the two groups. When cholesterol feeding ceased these high serum levels dropped. But the drop in the cholesterol content of serum and aorta were *significantly* greater in the animals fed vanadium.

Normal cholesterol levels in rabbits were:

Serum	20–50 mg. per cent
Aorta	2.5–4.5 mg./g dry matter
Liver	4–6 mg./g. dry matter

In a group of normal healthy young men similar treatment with vanadium resulted in small but significant lowering of the serum cholesterol [77].

While elements such as manganese and chromium increase cholesterol synthesis in rat livers, vanadium and iron decrease it [76]; vanadium appears to be unique in its ability to inhibit the utilization of mevalonic acid in cholesterol synthesis [24], and this is probably the main cause for its anti-atherosclerotic effect.

Hypertension

Another contemporary health problem is the widespread existence of hypertensive states. Manganese appears to play an important part in these conditions [71], while Schroeder and Perry [258] claim that 'all

human agents which are antihypertensive in man and animals, and which do not block the autonomic nerves . . . are characterised by an affinity for certain trace metals'. They showed that chelates and manganese were useful in removing hypertensive states in rats, but this effect was not *specific* to manganese, as other metals also possess it. The effect of the metals is not known, although it has been established that hypertension cases excrete high levels of trace elements in their urine, and that chelating agents restore this excretion to normal, together with the restoration of a normotensive state [221].

Diabetes and zinc

That a relationship exists between insulin and zinc has been known since it was shown that zinc combines with insulin to form crystalline insulin [271]. Further work showed the relationship between insulin and zinc in the bovine pancreas, the organ which is responsible for insulin synthesis [272]. Normally there is a high level of zinc in the pancreas, but in a diabetes mellitus this level is greatly reduced.

Studies show that zinc and insulin fluctuate in a similar way in experimentally produced diabetes, and substances that reduce the zinc content of the pancreas are, in fact, diabetogenic [154].

Zinc injections in diabetes are not beneficial, and result in a rise in blood sugar, and high blood sugar, as well as the excretion of large quantities of sugar in the urine, are symptomatic of diabetes. The injected zinc is not excreted, and it appears to be accumulated. This may result in fibrosis of the pancreas. There is no relationship between blood-plasma zinc levels and the duration of the diabetes [227].

Recently a report on the beneficial influence of an infusion of *Medicago sativa* (lucerne or alfalfa) has been reported in an atypical case of diabetes. Drinking this infusion resulted in a precipitous drop in blood sugar. Lucerne is rich in manganese, and it was shown experimentally that drinks of manganese chloride solutions, but not of sodium, magnesium, cobalt, iron, or zinc salts, brought about this dramatic drop in blood sugar [249].

Phalaris staggers

A condition in cattle and sheep termed 'Phalaris staggers' is of interest, as it shows the need for cobalt as a detoxicant. Animals grazing on pastures of the grass *Phalaris tuberosa* growing on cobalt-deficient soils suffered from muscular tremors, inco-ordination, rapid breathing, and pounding of the heart. This disease could be prevented by oral administration of cobalt, or by applying it as a top dressing to the pastures [83, 166] at the rate of 4-5 oz. cobalt sulphate per acre.

Phalaris contains an unidentified myelin destroying neurotoxic substance, or some precursor of one. The cobalt either prevents the

formation of this substance or is involved as part of a detoxifying system in the digestive tract. Injected cobalt does not help the animals.

A similar condition which shows the same response to copper cobalt is found in South Africa in animals grazing Ronpha pastures [309]. Little is known about the details of this condition.

Disturbances of trace-element metabolism in some diseases

A number of diseases are accompanied by serious disturbances of the metabolism of trace elements, although they are not necessarily due to nutritional factors. The fact that blood copper rises very appreciably in fevers and even in schizophrenia, as well as in pregnancy, has already been mentioned (page 115). But in Wilson–Usman’s disease (hepatolenticular degeneration) the body absorbs a very large percentage of the copper in the diet (healthy people only take up 5 per cent), so that, in spite of the increased excretion of copper, large quantities of copper are deposited in the tissues. The copper content of the brain is increased ten-fold [59, 74].

In haemochromatosis enhanced iron uptake takes place, just as copper does in the Wilson–Usman disease. Patients can absorb 20–45 per cent of the iron supplied in the diet, as opposed to 1–4 per cent in normal subjects. As a result, very large quantities of iron can be deposited in the liver of the patients [306].

On the other hand, in beri-beri, a thiamin (vitamin B) deficiency of man, Eggleton [96] showed a marked *decrease* in the zinc content of blood, hair, skin, and nails of sufferers, which was correlated to the deficiency. The pronounced drop in the zinc content of the prostate gland in cancer has already been discussed [182].

Psychological phenomena and trace elements

Organic illness, malnutrition, and excessive physical strain all result in psychological alterations in man and animals. Diet governs mental health to a considerable degree, so it is not surprising that *essential nutrients* should play some part in psychological processes, although this is seldom stressed. Yet trace elements are known to be involved in some very marked alterations of behaviour.

In the chapter dealing with reproduction lack of copper, iodine, or manganese was shown to result in a pronounced decrease in sexual urge or libido, although in the iodine deficiency sperm formation was normal [14]. In female animals lack of oestrus does not imply inability to ovulate, at least in iodine-deficient cows or manganese-deficient sows, but the lack of interest in intercourse consequent upon deficiencies results in great economic loss to the farmer.

Perhaps in this context the effect of high levels of iodine or manganese is of particular interest, as they result in symptoms among



FIG. 54

Cretins

Cretinism is a condition caused by the inability of the thyroid gland to produce the iodine-containing hormone thyroxine. The two cretins shown with a normal healthy man are dwarfed in stature and abnormal in development, as well as being completely retarded mentally.

which nymphomania is very prominent [12, 117]. In thyrotoxicosis (Grave's Disease) of humans the high levels of thyroxine produced result in 'a type of woman who is volatile, lively and temperamental, and who, if the disease is not too far advanced, is usually distinctly attractive to the male' [11].

Cretinism is a pathetic condition in which the thyroid gland is atrophied, and it is thus a condition produced by lack of the iodine-containing hormone thyroxine, rather than by a simple lack of iodine. Cretins, who are malformed and mentally sub-normal individuals (see Fig. 54), are found most commonly in goitrous areas. They can be helped considerably by thyroxine injections, which result in very striking improvements in their intelligence and cheerfulness, and enable them to lead a useful life [11].

Toxicities of various metals very frequently involve considerable psychological abnormality. The consequences of manganese toxicity in man have been studied in some detail in Chile [259]. As well as causing hypertonia, tremor of the extremities, impaired hearing, and giving a characteristic gait, the psychological alterations were very great. 53 per cent showed emotional instability, 47 per cent apathy, 40 per cent hallucinations, and 27 per cent disturbances of libido. The patients, who were miners, were suffering from severe manganese toxicity which was not due to excess of manganese in the diet.

Toxicities of non-essential elements are also well known, and of these, mercury poisoning is perhaps the most interesting in this context. It results in people being very prone to blush in embarrassment (erethism), apt to lose self-control, and being timid and easily discouraged [74].

In Wilson-Usman's disease, with its pronounced upsets of copper metabolism, there is a loss of conceptual thinking ability, and hallucinations are often present. In some cases there is real evidence of schizophrenia. These symptoms occur with the abnormal copper levels, but there is no evidence that they cause them [74].

Susceptibility to disease and trace elements

The fact that badly nourished plants, animals, and men are more prone to many kinds of disease is well known not only to farmers and doctors but also to gardeners and mothers. It might be expected, therefore, that imbalance of the micro-nutrient elements would also influence susceptibility to disease. While it is known that this is, in fact, the case, this aspect of trace-element nutrition has hardly been studied in any detail. In the case of major elements, such as potassium, low levels are known to predispose plants to fungal infection [88, 135].

It has been known for a long time that infections of *Phoma betae* are virtually confined to boron-deficient sugar beets [135]. In Table 31 a number of observed correlations between deficiency and parasitic

infections are shown, but as these are based upon incidental observations rather than on direct experimental investigations, they must be accepted with caution.

TABLE 31

Relationship between low levels of certain trace elements and enhanced susceptibility to infection in cultivated plants

ELEMENT	HOST	INFECTING ORGANISM
Boron	Barley	Mildew, <i>Erysiphe graminis</i> D.C.
	Wheat	Rust, <i>Puccinia triticina</i>
		Rust, <i>Puccinia glumarum</i>
	Sunflower	Mildew, <i>Erysiphe cichoracearum</i> *
	Beet	Mildew, <i>Phoma betae</i>
	Cauliflower	Mildew, <i>Botrytis</i> sp.
	Flax	Mildew, Bacterial infection
Copper	Wheat	Rust, <i>Puccinia triticina</i>
Manganese	Oats	Bacterial infection
Molybdenum	Lucerne	Lowers resistance to infection in general
	Lettuce	
Zinc	Rubber	<i>Oidium heveae</i>
		<i>Phytophthora</i> sp.

* Sunflower infection by mildew is used as an indicator of boron deficiency in the U.S.A. [57].

It is not likely that a condition exists in which the plant is resistant to all infections. Nor is it likely that a state which is resistant to one infection will not be susceptible to another. Thus, the fact that boron deficiency in barley resulted in heavy *Erysiphe graminis* D.C. (mildew) infections, but virtually none by *Helminthosporium sativum* is not surprising [95]. Williams [330], in a review, cites several cases where high, but not toxic, levels of nutrients decreased resistance to certain fungal infections.

Gäumann [112] draws attention to another interesting aspect of infection. The fungus *Fusarium oxysporum* f. *lycopersici* infects tomatoes and causes a characteristic wilting. This wilting is caused by a dipeptide, lycopersamin, whose activity is enhanced by the addition of ferrous salts. The lycopersamin can produce wilting only if it forms a complex with iron, but not otherwise.

Virtually nothing is known about the influence of micro-nutrient status upon virus infection, but Welkie and Pound [327] observed that tobacco mosaic virus infection was much more intense in manganese-deficient tobacco plants than in normal ones. De Kock [85] states that certain virus diseases bring about symptoms of iron deficiency. In this context it is worth recalling that the trace ele-

ments can alter the amino-acid content of plants appreciably (cf. page 70), and that virus infection brings about very similar changes [126]. There is a possibility that the viruses are involved in the same biochemical processes in which the trace elements participate, and that they may influence these reactions by altering the availability of these elements in the cells.

Animals

Very similar phenomena may be observed with animals. As Hopkirk and Patterson [137] point out, cattle and sheep suffering from latent as well as visible cobalt deficiency are usually heavily infected with worms. In fact, 'pining' was at one time considered as primarily due to parasitic infection. Cobalt in the diet makes worm control a relatively easy matter. In Russia sheep grazing on cobalt-deficient pastures showed severe lung infection by gram-negative cocci, and treatment of the sheep with cobalt resulted in a greatly reduced incidence of infection by this bacterium [158]. In Florida 'salt sick' cattle (a dual copper and cobalt deficiency) had chronic hookworm infection [206].

Copper deficiency has been found to be associated with heavy lung-worm infections, with severe trichostrongylosis (worm infection) and other internal parasite infections [48]. However, in experiments in Queensland copper deficiency was not found to be a major factor in determining susceptibility to parasitic gastro-enteritis [48].

In Germany oral applications of trace elements were successfully used to treat vaginitis in cows [292]. In America cows suffering from brucellosis (Bang's bacillus infection) were fed on fodder produced on trace-element fertilized soils. They are reported to have recovered completely and to have given birth to healthy calves. Similarly infected animals fed on ordinary fodder did not recover [26].

Hookworm anaemia in man

Worm infections in man can result in serious anaemias. The cobalt deficiency caused by fish tapeworms in Finland has already been described [214], and the manner in which it accumulated cobalt at the expense of the host resulted in severe anaemia.

However, the hookworm anaemia investigations of Cruz in Brazil are of particular interest, not only because it is such a widespread disease in the tropics but because he clearly demonstrated that hookworm anaemia is primarily an iron-deficiency disease, and must be treated as such [73].

Hookworm anaemia is found mainly in the central and northern parts of Brazil, where the diet is farinaceous. It occurs almost exclusively among those members of the population who cannot afford meat, and the wealthier members of the community do not suffer

from it. Yet worm infection is found in all groups, but it is only those on diets poor in iron who develop anaemia. In the south of Brazil, where the population is mainly meat eating, this anaemia is rare, although worm infection is rife. The slight haemorrhages caused by the worms do not result in anaemia and ill health if the iron intake of the body is adequate. It is only when the iron nutrition is inadequate that anaemia is found. Clearly, the lack of iron rather than the parasite is the main problem.

This rather protracted chapter involving so many different facets of the health problem shows again how varied the processes are in which trace elements are involved, and of what importance these essential elements are in the regulation of health and well-being. The consequences of imbalance give an insight into the complex integrations that are essential if health is to be maintained.

IX | The prevalence and distribution of trace element imbalances

Geographical extent

If imbalances of trace elements were rare in nature, and deficiencies or excesses only occurred very infrequently, then they would still be of interest to scientists, but would be of very slight practical importance. But in actual fact these conditions are found very extensively and are recorded from all parts of the globe. They result in considerable economic losses, as well as ill health in plants, animals, and man. It is estimated that nearly 200 million people suffer from endemic goitre [148], while in a small country like New Zealand, if no cure had been found for cobalt deficiency a million breeding ewes worth £18 million would have been lost [80].

Due to lack of information, it is not generally appreciated how extensive the areas affected by imbalances are. Except for goitre, which is not always a simple iodine-deficiency condition, these conditions have not been studied on a global scale. It is only in a few areas that information relating imbalances to soil types, climates, and other environmental factors is available. With the increase of *induced* deficiencies, a problem that will be discussed in Chapter XII, a greater interest is being shown in plant-soil-animal inter-relationships, with very interesting results.

In this chapter information is produced to show that, even at our present state of knowledge, there is evidence to show there are very widespread areas of deficiency in all the continents. It is quite obvious that imbalances are not rare, and that they must be looked upon as one of the common environmental factors that are likely to be found in any part of the world.

Goitre

As has already been mentioned, simple iodine deficiency is one of the main causes of goitre, but its effects may be magnified by many environmental, nutritional, and physiological factors. No other nutritional trace-element imbalance has been so intensively studied and its distribution so well mapped [148]. Its world-wide distribution is clearly shown in Fig. 55.

Sea-water and marine plants and animals are a rich source of

iodine, and this has led many people to believe that distance from the sea was an important predisposing factor in goitre. In actual fact, coastal areas may be very goitrous, as can be clearly seen in Fig. 56.



FIG. 56

IODINE INFORMATION [13]

Iodine-deficiency areas in the British Isles

The fact that iodine deficiency can be found in maritime climates is clearly depicted in this map. The deficiency occurs along the sea coasts, as well as inland, clearly demonstrating the fallacy that iodine deficiency and goitre are not found in coastal areas.

In Britain, where no region is more than 50 miles from the sea, goitre is by no means rare, and the Medical Research Council have recommended the introduction of iodized salt [186].

Nor is goitre confined to humans. Animals, especially cattle and

sheep, suffer from 'swollen neck' on a large scale. The seriousness of this deficiency can be gauged when it is appreciated that in Montana, one of the states in the Goitre Belt of the U.S.A., *1 million pigs died annually* before iodized fodders were introduced [13].

Certain types of rocks seem to favour the occurrence of goitre. Carboniferous Limestone is the most notorious of these, but it is not invariably linked with iodine deficiency. The Alpine Limestones (dolomite formations) of Northern India are the parent rocks in areas of severe goitre. In Britain at least chalks are not associated with severe deficiency. Sedimentary rocks like Old Red Sandstone have a tendency to give rise to goitrogenic soils, while igneous or metamorphic rocks, such as granites and basalts, rarely do.

Extensive deficiencies

Spectacular though the iodine picture is, there is no reason for believing it to be unique. Oram [216] estimates that 50 million acres (approximately 77,500 sq. miles) of crops require boron fertilization, and only one-quarter are getting it. In Australia, well known for its trace-element deficiencies, about 300 million acres (approximately 388,000 sq. miles) of adequately watered land is undeveloped agriculturally, primarily due to lack of trace elements. These regions can be as readily reclaimed as the zinc- and copper-deficient Ninety Mile Desert, which is now a flourishing farming area [8]. This is over four times the present agricultural area of Australia.

The nature of deficient areas

It must be remembered that not all animals and plants have the same mineral requirements, and healthy and deficient species can, in fact, grow side by side. Frequently the adapted local flora and fauna are healthy, but imported domestic plants and animals show signs of malnutrition. In this book the term 'deficient area' is used to denote a region in which deficiencies can be readily found, but it does not imply that all plants or animals in that area are, in fact, deficient. It does not even imply that certain elements are in very short supply, because, as will be seen later, imbalances can be *induced* by unsuitable agricultural practices, or even by climatic conditions.

As trace-element disequilibria are very varied, and it is desirable to grasp the extent of these conditions, it is perhaps most convenient to look at the known disturbances in each continent.

Europe

Trace-element deficiencies are not confined to poorly developed areas. Even in Holland, which is probably the most efficient agricultural country in Europe, acute trace-element problems exist, and Lehr

[168] claims that half of Holland is potentially deficient in trace elements. 'Infertility' in cattle has become widespread, and extensive deficiency of manganese [116] and copper [254], most of it induced, has been recorded.



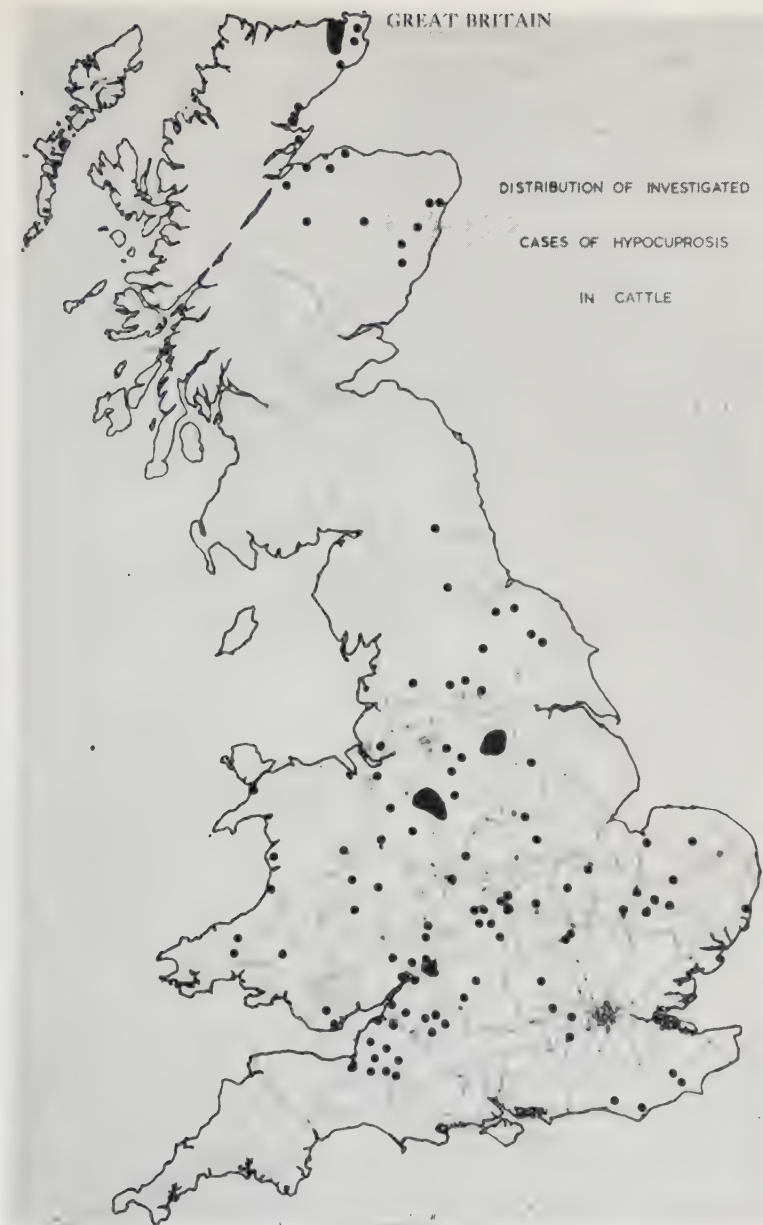
FIG. 57

FROM SCHLICHTING [254]

Copper deficiency in North-West Germany

This map shows the wide distribution of this deficiency. It is of particular importance to note that this condition appears to be spreading, as the distribution of the 1950-54 survey shows.

The North German Plain, which stretches from the Baltic Sea, across Germany and Denmark, into Holland, is a region that is copper deficient [230]. These are mainly humus-rich sandy soils. Fig. 57 gives some idea of the distribution of this deficiency in North-West 136



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FIG. 58

Copper deficiency of cattle in Britain
Distribution of investigated cases of hypocuprosis in Britain.

Germany, and it is important to observe that the deficiency has become more widespread since 1928.

Boron deficiency, especially in sugar beet, is very widespread, especially on tundra and moor soils [158], and in Latvia about 10 per cent of the annual crop is a total loss due to lack of boron [216], while in England half the swede acreage has boron deficiency, and 50 per cent of the potential Moselle grape crop is lost due to the same cause [216].

As well as these very large areas, more localised deficiencies are known. In Spain peaches and pears are widely deficient in iron,



FIG. 59

COPPENET [69]

Distribution of manganese deficiency in North-West France

This condition appears to be spreading due to excessive applications of marl and shell sand fertilizer very rich in calcium carbonate, and is a cause for concern, as this deficiency can result in considerable losses to the extensive dairy industry of this area.

copper, and zinc, and potatoes in manganese [239]. In Switzerland and Italy simple boron deficiencies are found [216], while in Germany, in addition to the copper and boron deficiencies mentioned, manganese, molybdenum, and cobalt deficiencies are found [325, 163, 254].

In Britain and Ireland, as well as boron, deficiencies of iron and manganese are encountered in fruit trees [300, 320] and copper in animals, as is shown in Fig. 58 [2], and in plants [250].

In France the spread of manganese deficiency is cause for concern cf. Fig. 59 [69], and in Russia copper deficiency is found in the North Caucasus [157], while in Latvia lack of cobalt constitutes a serious problem [158].

As well as deficiencies, excesses also constitute a serious problem. Although no really extensive areas of trace element toxicity are recorded in West and Central Europe, the heavy copper spraying carried out to combat the fungal infections over the last eighty years or so has resulted in high soil copper contents and toxicities have been recorded in the vineyards of France [334] and Germany [108].

America

Trace-element deficiencies are as widespread in the Americas as in Europe, but it is only from the U.S.A. that sufficiently large-scale investigations provide information to show how extensive these are. Fig. 55 (page 133) shows that extensive goitre belts exist both in North and South America and, as has been mentioned, the iodine deficiency is acute in some of these regions, as, for instance, in Montana. The conditions as known in 1958 in the U.S.A. were mapped by Thacker and Beeson [298], and this map (Fig. 60) shows extensive deficiencies of manganese, copper and zinc. In plants, boron deficiency is found particularly in plants growing on the Atlantic coastal plain, the north-west Pacific coastal region, Tennessee and North Carolina, and a belt running from Michigan through Wisconsin to Minnesota. The zinc deficiencies are also extensive, being found throughout the cotton belt, on the Pacific coast, and in Wisconsin. Manganese deficiencies are especially acute in Florida, but found in the muck soils of the Great Lakes Region, as well as in Southern California and the Atlantic Coastal Plain, while that of copper is found in the Great Lakes Region, Washington, South Carolina, Florida, and California [299]. This and other work, such as that quoted by Bear [28], makes it clear that these conditions are more extensive than was formerly suspected.

The Central American states are also known to suffer from these problems. In Mexico boron, iron, manganese, and zinc deficiencies have been identified, especially in fruit trees [184]. In Costa Rica, where good-quality coffee is produced, there are more serious trace-element deficiencies than in major elements. The central plateau is severely boron deficient, and application of this element gives yield increases of over 250 per cent [115].

In South America the iron deficiency in humans has already been discussed [73]. In the state of São Paulo severe cobalt deficiency exists in cattle [70], and zinc and magnesium deficiencies occur widely in crops. Boron, copper, and molybdenum deficiencies are also known. Although there is less information available from the other parts of Brazil, deficiencies have been reported from the Amazon in the north to the southern boundaries [265].

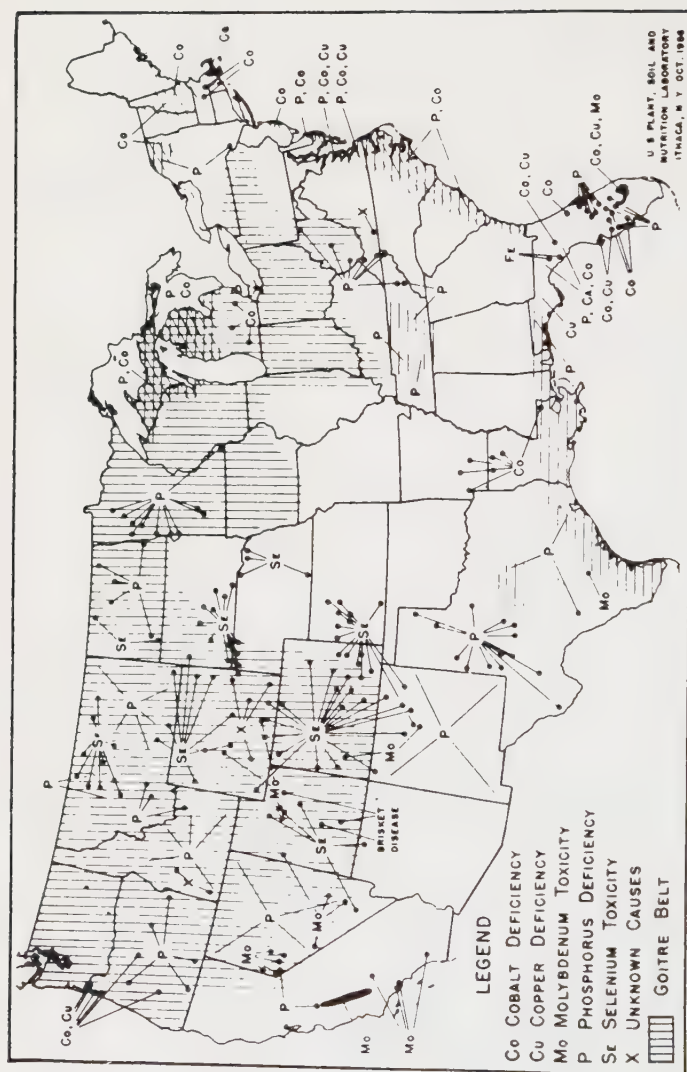


FIG. 60

FROM THACKER AND BEESON [298]

Mineral-nutritional diseases of animals in the U.S.A.

Known areas in the United States where mineral-nutritional diseases of animals occur. The dots indicate approximate locations where troubles occur. The lines not terminated in dots indicate a generalized area or areas where specific locations have not been reported.

Africa

Trace-element imbalances of various kinds constitute a serious agricultural problem in Africa, affecting plants, animals, and man. As large-scale systematic agriculture has been practised in most areas south of the Sahara Desert only in very recent times, the type of information readily obtained in areas such as Europe could not be expected to be easily available. A survey published in 1955 [261] made it quite clear that deficiencies existed on a very large scale, cf. Fig. 61. Since this time many more have been identified and successfully combated.

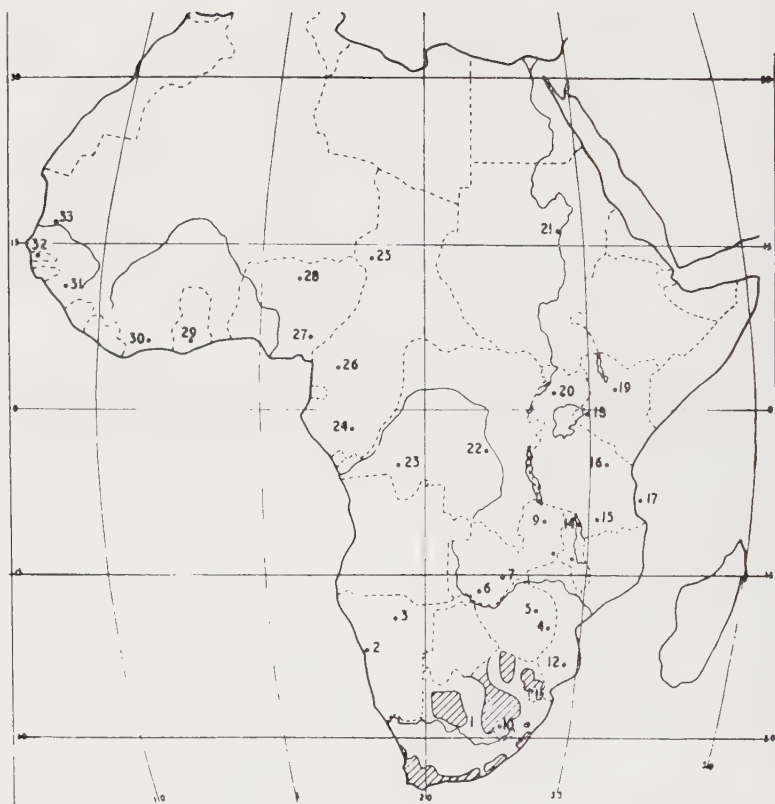
In the Republic of South Africa, where these conditions have been more intensively investigated, it would appear that about 80 per cent of agricultural land is affected. Swayback and steely wool (copper deficiency) are found on the coastal plains north of Cape Town. In 1950 in the deciduous-fruit-growing areas of the Western Cape Province about 3 million out of a total of 8 million bearing fruit trees were affected with trace-element deficiencies [36]. Copper, zinc, and manganese deficiencies are widespread, and now lack of molybdenum and boron are also being discovered [35, 36].

In East Africa recent work has brought to light extensive deficiencies in crops and animals [58, 243], while many are known from the Congo [165, 256]. On the other hand, trace-element deficiencies do not appear to constitute a serious problem in Ghana, but they are known to exist there [129, 164], and also in other parts of West Africa [131, 324].

In general, trace-element deficiencies are widespread throughout Africa in crops and to a lesser extent in animals, and they have been found in all larger regions where they have been looked for.

Australia

Australia is undoubtedly the continent in which acute deficiencies are most widespread. As the agricultural problems are so extensive, it is not surprising that Australian research work in this branch of science, as well as its practical application, leads the world in many respects. The spectacular results obtained by curing the zinc and copper deficiency of South Australia's Ninety Mile Desert, which have resulted in this area being re-christened the Coonalpyn Downs, and the development of the 3-million-acre Esperance Plain in Western Australia have already been mentioned [8]. The podsollic soils of eastern Australia are nearly all more or less molybdenum deficient. They stretch for 1,000 miles along the coast, and are also found in Tasmania; the belt is often more than 150 miles wide, running through the populous states of Victoria and New South Wales up to Queensland. In Figs. 62 and 63 the nature of the change resulting



1. Union of South Africa, see previous maps.
2. Swakopmund (S.W.A.), Fe and Mn.
3. Tsumeb (S.W.A.), Fe and Mn.
4. Umtali and Eastern Districts, (S.R.), Zn, Mn, Mg, B, Mo.
5. Mazoe, (S.R.), Zn, B, Cu (?).
6. Choma and Kaloma areas, (N.R.), Mg, Cu, B, Zn.
7. Lusaka area, (N.R.), B, Zn, Mg.
8. Fort Jameson, (N.R.), B.
9. Shiwa Nganda, (N.R.), Zn, Mg.
10. Basutoland, Mf.
11. Swaziland, Zn, Mn, B, Mg, Cu.
12. Moçambique (P.E.A.), B, Mn.
13. Nyasaland, Zn, Cu (?).
14. Nyasaland, S.
15. Tanganyika, S, Zn, Fe.
16. Tanganyika, Zn, Mg, Mn, Cu (?).
17. Zanzibar, Co, Cu, Fe, Mg, Mn, Zn.
18. Kenya, Cu, Mg.
19. Kenya, Cu (?), Zn (?), S (?).
20. Uganda, Zn, Mg, Mo (?), Fe (?), Cu (?), S (?).
21. Sudan, Zn (?).
22. Belgian Congo, Cu.
23. Belgian Congo (Kasai area), B.
24. French Equatorial Africa, Mg.
25. French Equatorial Africa (Chad area), Zn.
26. French Cameroons, Zn, Mn (?).
27. Nigeria, S.
28. Nigeria, S.
29. Gold Coast, S.
30. Ivory Coast (F.W.A.), B, Fe.
31. Guinea (F.W.A.), Zn, Mg.
32. Gambia, Cu, Mg, Mn, B.
33. Senegal (F.W.A.), Mg, Cu, Mo.

from correction of this deficiency is shown. This deficiency is more complex than a simple molybdenum deficiency, and the best results in the field are obtained by applying molybdenum with a superphosphate fertilizer. The doses of molybdenum are so minute, 1–2 oz. of sodium molybdate per acre remaining effective for six years [6],



FIG. 62

FROM ANDERSON AND UNDERWOOD [8]

The type of infertile land that is being converted by copper and molybdenum fertilizers into arable land in Australia

Such tracts of land were virtually worthless until scientists discovered that lack of small amounts of copper or molybdenum were limiting production. Once the deficiency is cured, the land is of value in agriculture (see Fig. 63).

that a real problem exists in distributing such small quantities evenly over the lands.

The severe copper deficiency of parts of Australia, especially in the south and west, resulting in widespread sheep and cattle disease, as did the combined copper-cobalt deficiency, which was found mainly in the south [180], have been recognized and effectively treated today, with a consequent marked expansion of agricultural land.

FIG. 61

FROM SCHÜTTE [261]

The distribution of micro-nutrient deficiencies in Africa

The numbers indicate the areas and crops in which deficiencies occur. Considering the paucity of research centres in Africa, this map indicates a very widespread distribution of these conditions, which more modern work has substantiated.

Asia

Although Asia is the largest continent, it is not possible to assess the significance of trace elements in it. Very extensive iodine deficiencies exist, and boron is widely deficient in Japan and South-East Asia [216]. In India, where climatic and soil conditions may be expected to result in deficiencies, apparently conflicting evidence exists. In Bombay state there have been spectacular responses to copper in rice crops [142]. In Delhi applications of boron and of molybdenum have significantly improved the yields of berseem (*Trifolium alexandrinum*) [81], and sugar cane shows some beneficial effects from various trace-element sprays in the Punjab [231]. Soil analyses tend to indicate that



FIG. 63

ANDERSON AND UNDERWOOD [8]

Reclaimed farmland in the Southern Tablelands of Australia

This land was infertile due to a lack of molybdenum and had the appearance of rough country as shown in Fig. 62. The establishment of this farm was possible only because the molybdenum deficiency had been combated by fertilizers.

soil contents of some of these elements appear to be adequate in the areas studied [207]. Certainly in Ceylon copper deficiency is known on certain of the tea estates [232]. In Malaya, one of the big rubber-producing areas of the world, iron and manganese deficiencies are known in the field [42].

All told, the evidence in the readily available literature does not enable Asiatic conditions to be discussed, although there is no reason for believing that conditions there differ from elsewhere in the world.

Toxicities

No discussion concerning the distribution of deficiencies would be complete without reference to areas of excessive or toxic levels of these elements. These also occur, although on a very much more restricted scale. Locally, they may be quite extensive and the cause of

serious agricultural or health problems. Perhaps the most widespread of these conditions is excess of fluorine, which causes 'fluorosis' of stock and of man (see page 93). It is fairly extensively distributed in the U.S.A.; in North Africa it is 'darmous' disease found in cattle, sheep, horses, and man; in Tanganyika and South Africa large areas are afflicted [306]. It is worldwide in its occurrence, and in its most extreme form is found in the Madras Presidency, where 'poker-back' occurs [279]. Excess fluorine accumulates in the bones, and in severe fluorosis osteosclerotic symptoms develop, and thickening of the joints occurs. In really severe cases the vertebrae of the back may form a rigid structure, giving rise to 'poker-back'. Mild cases take the form of mottled teeth. In animals malformation of the bones and teeth may result in poor growth, as the animal finds feeding very difficult.

In the semi-arid regions of the Great Plains of North America animals suffer from a disease termed 'alkali disease' or 'blind staggers'; it is frequently fatal. This has been shown to be due to selenium poisoning, which is prevalent in these soils. It is accentuated by the existence of accumulator plants such as *Astragalus* spp. which concentrate the selenium and increase the toxicity of the herbage. This high level of selenium results, particularly, in badly deformed hooves (Fig. 7, page 37). Monstrosities are also formed under these conditions. There is no definite evidence that human disease results from these soils, probably because human eating habits are such as to make man far more independent of the immediate surroundings, his food coming from many dispersed areas, and so toxic concentrations do not accumulate in the body.

Boron toxicity is more restricted in its distribution, and is often induced by poor agricultural techniques. In dry regions unwise irrigation can result in salts crystallizing out of the water, and 'brack' conditions can be formed. Borates separate out and may poison the vegetation. In California this has been shown to occur [216]. Certain plants which have a very low boron requirement, such as many grasses and cereals, certain grain legumes, rubber, tea, and cocoa, are very sensitive to high levels, and readily show toxicity symptoms.

More localized toxicities also exist in many areas to cause practical problems, manganese toxicity exists in the coffee plantations of the Kivu (Congo) [256] and in parts of South Africa [37].

Toxicities of non-essential trace elements such as cadmium, nickel, and aluminium are known and result in abnormalities of plants and of animals and men. Kobayashi [122, 153] reports a very severe poisoning of a population as a result of a mine polluting river water with cadmium salts in Japan.

These toxicities are far more difficult to treat than deficiencies, so it is fortunate that they are not so extensive as deficiencies, even though they are widely scattered.

X | Ecological aspects of trace elements

Adaptation to special environments

Not all animals and plants have the same requirements, and some can survive, and even flourish, in habitats that are quite unsuitable for most organisms. These plants and animals that can survive in these regions are specially adapted to these conditions. Just as brack soils have a specialized flora growing on them, so copper-deficient regions have a characteristic vegetation. In the case of poisoned soils and ore dumps, a very specialized group of plants can inhabit these successfully. In Roros in Norway *Viscaria alpina* and *Melandrum dioicum* grow profusely on cupriferous sulphide deposits. Other plants that can tolerate these conditions range from small trees to mosses. Table 32 shows a number of these adapted plants [313].

TABLE 32

Adapted plants growing on cupriferous sulphide deposits in Norway.
From VOGT [313]

<i>Equisetum arvense</i> L. (horsetail)	<i>Eriophorum polystachion</i> L.
<i>Equisetum palustre</i> L. (horsetail)	<i>Carex rostrata</i> Stokes (reed)
<i>Equisetum limosum</i> L. (horsetail)	<i>Salix reticulata</i> L. (willow)
<i>Juncus trifidus</i> L. (sedge)	<i>Salix herbacea</i> L. (willow)
<i>Agrostis canina</i> L. (grass)	<i>Salix glauca</i> L. (willow)
<i>Agrostis borealis</i> Hn. (grass)	<i>Salix arbuscula</i> L. (willow)
<i>Betula odorata</i> (birch)	<i>Oligotrichum hercynicum</i> (Hedw)
<i>Betula nana</i> L. (birch)	Lav. (moss)
<i>Eriophorum vaginatum</i> L. (bog cotton)	

The fact that special adapted floras exist in many parts of the world is particularly stressed by Russian workers. The concept of the biogeochemical province introduced by Vinogradov is of practical significance. The characteristics of, say, a boron-deficient province can be studied and measures taken to combat agricultural or health problems that exist [157, 158, 311]. This concept stresses the soil-plant-animal-man inter-relationships that are so important, i.e. biocycles.

Adaptation can be of two kinds, either genetically or physiologically determined. These are not mutually exclusive groupings, and phy-
146

biological factors can vary the expression of a genetically predetermined adaptation.

Genetic adaptation

The specific differences between plants are genetically determined, as is the ability to tolerate apparently toxic levels of certain elements, as in the case of mine-dump floras. In Britain Bradshaw and his co-workers have shown what a large tolerance range can be found within a single species [45, 143, 144]. The adapted strains must be due to a natural selection of genetically mutated populations, for their nutritional responses can be quite distinct from plants of the same species growing on normal soil in their immediate neighbourhood. Table 33

TABLE 33

The response of populations of the grass Agrostis tenuis from 'lead soils' and from normal pastures to calcium and phosphate applications, expressed in grams of dry matter produced. From JOWETT [144]

POPULATION	CALCIUM, P.P.M.			
	5	15	50	200
Lead mine	53.21	50.51	50.24	47.45
Pasture	53.42	77.65	70.43	58.94

POPULATION	PHOSPHORUS, P.P.M.		
	5	25	125
Lead mine	52.89	74.74	73.78
Pasture	66.80	93.22	100.42

The populations from the 'lead soils' show very little response to either calcium or phosphorus, although the ones from normal pastures do. The populations from the 'lead soils' are adapted to high levels of lead, copper, and nickel, and low levels of calcium and phosphate.

This type of adaptation is probably more widespread than is generally recognized.

gives some quantitative information about responses of the grass *Agrostis tenuis* to both calcium and phosphate application in such a case [144]. In spite of the numerous environmental factors which altered the level of iodine in perennial rye-grass, Butler and Glenday [56] found that there is a genetic factor that determines iodine levels. Table 11 (page 64) also showed that in the copper uptake of wheat there is a marked varietal difference which must be genetically determined. So the state of affairs shown in Fig. 64, where oats are shown to respond well to copper applications, while the growth of mustard is depressed at the same concentrations, is of a genetical nature.

Just as tolerance to toxic levels of various elements is genetically determined, so sensitivity to even traces of others must be due to this. Fluorine is an element that most plants can tolerate in small doses,

and some even accumulate it. Camellia species tend to accumulate very appreciable levels of this element, and tea, especially China tea, may contain more than 100 p.p.m. fluorine [194]. Yet this same element, which is present as hydrofluoric acid in certain industrial waste gases, is so toxic to plants like gladioli, tulips, and maize, that these plants cannot be grown in the vicinity of certain manufacturing areas [336].

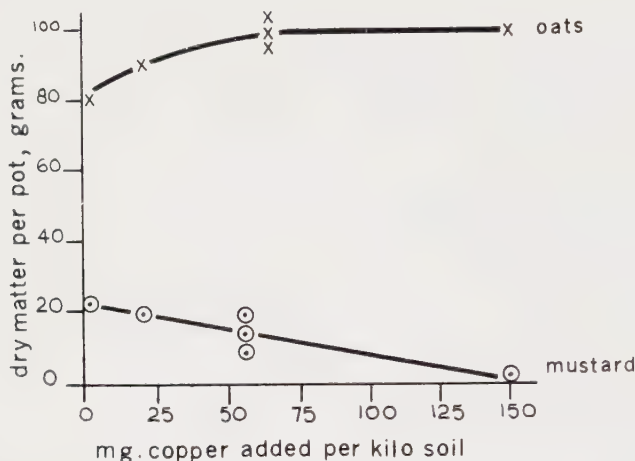


FIG. 64

KICK [151]

Response of oats and mustard to increasing applications of copper

This graph demonstrates the marked difference in response to copper in oats and mustard. The oats responded well to copper applications, and even at levels of 150 mg./kg. soil, no growth inhibition was observed. On the other hand, all additions of copper decreased the yield of mustard. The copper content of the mustard at the higher levels was two to four times that of the oats.

Physiological adaptation

The balance of trace elements in a plant regulates the bulk of the reactions in a plant. It is therefore not very surprising that these elements also play a part in regulating the water economy of the plant. It is well established that deficiencies of boron, copper, manganese, and zinc detrimentally influence transpiration [255, 264], and this is clearly shown in Fig. 65. Another observation of interest is that soil applications of boron resulted in a higher transpiration rate when water was freely obtainable, but this reduced water loss when water was not readily available [90]. This shows that *adequate supplies of boron result in plants being better adapted to dry conditions* and having a better xerophytic behaviour. Nor is this adaptation confined

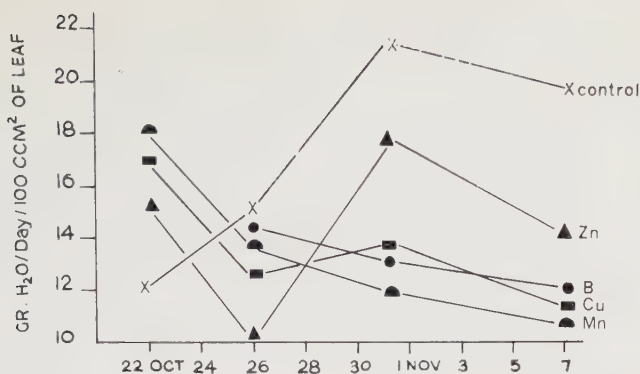


FIG. 65

FROM SCHÜTTE [264]

The influence of trace-element imbalance on the transpiration rate of the nasturtium (zinc, boron, copper, manganese)

All the deficient plants showed an appreciably lower transpiration rate than the control plants. This marked difference in water relations can be measured long before any visible signs of deficiency can be detected.

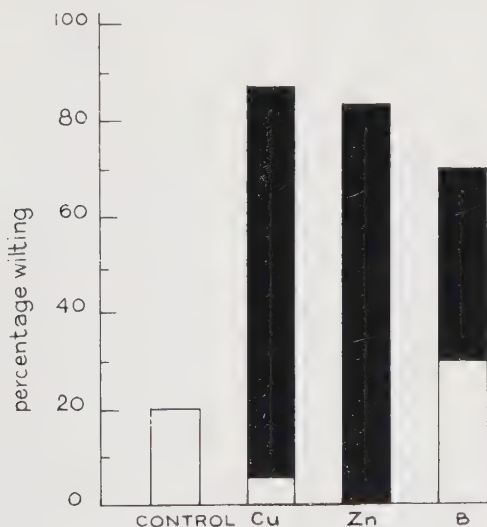


FIG. 66

FROM SCHÜTTE [263]

The influence of trace elements on the rate of wilting in radishes

The water relations of plants, including the rate of wilting, are influenced by trace-element nutrition. It is clear that radishes receiving full balanced mineral nutrition (control) are less prone to wilting than deficient ones. Zinc (Zn) and copper (Cu) deficient plants are particularly prone to wilting, while in early stages of growth boron (B) deficient ones are not so susceptible, but when mature are much more so.

to boron-sufficient plants, as copper and zinc deficiency result in plants being particularly prone to wilting [263], cf. Fig. 66.

That frost resistance can be altered by trace elements has been known since the 1920s, and by adequate applications of these elements, the frost resistance of a large number of plants was enhanced in German moorlands [103]. More recently both boron and copper have been reported to show this protective action in the Northern Transurals [290]. On the other hand, deficiencies of molybdenum and of manganese lowered the frost resistance of lucerne [43]. In contrast to this, heat resistance has been studied in the parts of the Volga exposed to intense summer heat, and enhanced levels of zinc are reported to increase the heat tolerance of the vegetation [222].

The influence of the environment on trace-element requirement

Climatic conditions are of great importance in the development of deficiency diseases. Long dry summers, in which the soils are dried out, result in widespread deficiencies of copper, boron and manganese. These elements are not readily available under the oxidizing conditions of a well-aerated and dry soil. In wetter seasons these deficiencies are not common. It must not be overlooked that the deficiencies produced by the dry spells are augmented by the fact that these deficiencies, which are induced, make the plants more prone to wilting and less adapted to survival in a dry climate.

Copper and manganese particularly are not readily plant available in dry soils, because these are more oxidizing than moist ones. But the presence of carbon dioxide in the soil can bring about reducing conditions, and this results in the soil manganese being made more available [41]. It also explains the benefits derived from the empirical practice of some farmers in industrial areas who spray their crops with soda-water (carbon dioxide super-saturated water). Waterlogging of the soil also results in reducing conditions being developed, and it can result in so much manganese being made available that toxicity may result.

Light is another factor of importance, and it is particularly associated with zinc requirements. Intensive illumination results in an increased zinc requirement, and often in an induced zinc deficiency. On fruit trees the side nearest the sun usually shows much severer deficiency symptoms than the rest of the plant. In fact, the relationship between zinc and growth hormone has been studied. Light inactivates the growth hormone β -indole acetic acid, and the zinc level controls the amount formed [66]. As this hormone controls the growth and normal development of plants, it is not surprising that light-induced zinc deficiency results in conditions such as 'little leaf', caused by lack of hormone, as with the rapid light inactivation, the plant's requirements of zinc are increased appreciably.

But other light effects are also found. Shade prevents injury to beans and tobacco grown on soils with toxic levels of manganese in them, and reduces their uptake of this element [172]. In coffee, shade results in an appreciable increase in manganese level, and a decrease in the important iron/manganese ratio. Shading increases the zinc levels and lowers the boron ones in general, compared to insolated plants [174], as can be seen in Table 34.

TABLE 34

The influence of light and shade on the trace-element composition of coffee leaves. From LOUÉ [174]

DATE	SHADE, P.P.M.						WITHOUT SHADE, P.P.M.					
	Fe	Mn	Zn	B	Cu	Fe/Mn	Fe	Mn	Zn	B	Cu	Fe/Mn
1 Sept. 1953	117	81	26.0	85	—	1.44	110	43	10.5	109	—	2.55
16 Oct. 1953	145	95	12.5	81	—	1.52	135	76	17.2	103	—	1.77
20 Nov. 1953	122	82	16.1	90	—	1.50	102	62	11.6	112	—	1.64
19 Jan. 1954	182	70	9.6	48	—	2.60	133	50	9.2	57	—	2.66
2 March 1954	192	86	18.4	58	18	2.23	128	54	13.5	48	18.0	2.37
20 April 1954	112	78	11.6	54	18	1.43	146	28	13.6	63	16.5	5.21
16 June 1954	144	78	18.6	70	17	1.84	108	40	15.0	56	19	2.70
27 July 1954	147	99	14.6	84	26.3	1.48	120	48	14.1	84	21	2.50
7 Sept. 1954	153	93	10.1	90	23.4	1.64	146	54	11.1	112	21.6	2.65
20 Oct. 1954	152	138	13.7	96	25.0	1.10	91	79	12.0	77	21.3	1.15
16 Nov. 1954	110	76	10.5	73	26.6	1.44	87	47	13.1	95	19.9	1.85
30 Dec. 1954	200	78	10.5	121	17.5	2.56	175	58	10.4	135	16.4	3.02
11 Feb. 1955	277	91	10.9	96	19.0	3.04	242	56	9.8	129	15.0	4.32
22 March 1955	175	89	9.8	196	18.0	1.96	125	65	10.7	96	14.2	1.92
29 April 1955	112	68	—	87	25.0	1.64	97	40	—	89	16.5	2.42
10 June 1955	132	85	—	113	20.5	1.55	105	58	—	112	14.9	1.87
22 July 1955	97	75	—	92	27.1	1.29	77	59	—	112	24.5	1.30

It is quite clear that light intensity is very important in regulating the trace-element content of coffee leaves. The manganese content of the leaves of shade-grown plants is much higher than those grown in full sunshine, but the important iron/manganese ratio is less, invariably. Zinc is more plentiful, and boron less so in the leaves of the shaded plants.

As well as light intensity, light duration is also of importance, as it regulates the photoperiod, i.e. time exposed to light, of plants. Some of these are very sensitive to changes in photoperiod. In certain soya-beans, buckwheat, and cockle-burr, boron deficiency only develops under long-day conditions, but not under short-day exposure to light. The boron requirement is enhanced by more than 14 hours daylight. Yet in plants such as tomatoes or sunflower the duration of the daylight does not alter the mineral requirements [293].

Temperature is also an important environmental factor, and beans can tolerate levels of manganese at high temperatures that would be toxic at lower ones [172].

Clearly, the environmental factors play a great part in mineral nutrition, but as yet there is very little experimental information available about this aspect of nutrition.

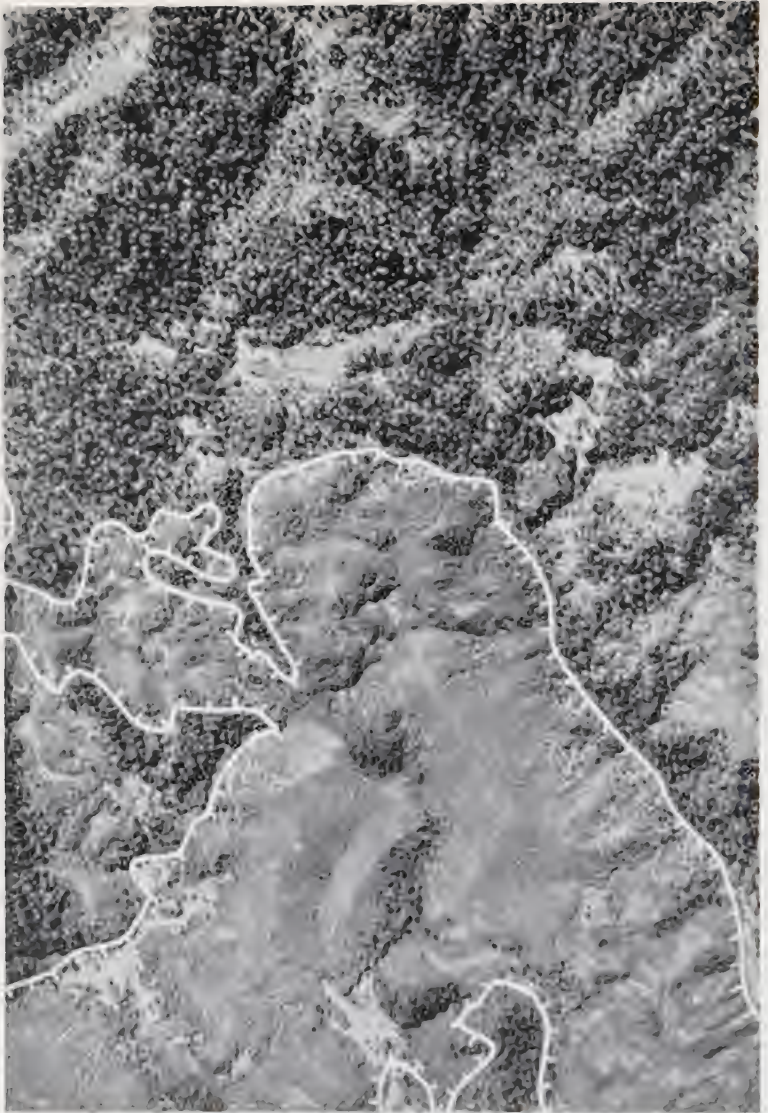


Photo by courtesy of Pacific Southwest Forest and Range Experiment
Station, U.S.D.A. Forest Service, California

FIG. 63

Serpentine Flora, a specially adapted flora

This aerial photograph from California shows how the nature of the vegetation is influenced by the soil type. The dominant vegetation is forest growing on sandstone, with such species as *Pseudotsuga men-*

Two examples of adapted floras

Enough has been written about the factors involved in adaptation, but what are these adapted faunas and floras like? Can they be recognized with any ease? The answer is that the degree of adaptability required for the specialized environment determines this.

The North German Plain is a fine example of an area that is known to be copper deficient, and has an adapted flora. This is a region of humus-rich, sandy soil, and the vegetation is such as might be expected in moorland areas. The plain is not absolutely deficient in copper, but the organic matter in the soil fixes the copper very readily, and makes it unavailable to most plants. In this environment the ability to thrive is closely linked with the ability of the plants to absorb copper under these conditions, and the characteristic heath plants of this region, such as *Calluna vulgaris* and *Erica tetralix*, contain appreciable amounts of copper. The same is true of other plants, such as birch and buckwheat, which are typical of this area [229, 230].

Local varieties of oats and barley, as well as rye and buckwheat, *Fagopyrum esculentum*, grow and yield harvests in these areas, but high-yielding strains of imported cereals succumb to deficiency diseases and yield no crops. This ability to grow normally, found in local varieties such as the oat Rotenburger Schwarzhafers, is due to the fact that they can absorb their copper requirements rapidly in their youth, before drought has made the soil copper unavailable to the vegetation. Imported varieties, such as Dippes Siegeshafer, absorb their copper very much more slowly, and at ear formation, when the need for copper is particularly great, there is little available in the soil. They cannot escape the detrimental effects of the summer droughts, as the local crop plants can.

This moor region of Germany is particularly suited for forests, and many plantations exist. But again, introduced species of trees, such as the spruce *Picea excelsa* and the larch *Larix decidua*, are unable to absorb enough copper for optimal development, while the indigenous birch *Betula verrucosa* is. In practice, applications of copper tend to limit its growth.

In many copper-deficient soils the organic matter is concentrated

ziesii, *Pinus ponderosa*, and *Quercus kelloggii* dominating the vegetation. But this vegetation cannot flourish on the Serpentine outcrop, whose limits are shown by the white line. Here a specially adapted flora, including species such as *Cupressus sargentii*, *Arctostaphylos viscida*, *Adenostoma fasciculatum*, *Quercus durata*, *Pinus attenuata*, grows on a soil on which most plants cannot thrive. The border between the two zones is sharp, and the nature of the vegetation gives a very good indication of the nature of the soil and bed rock upon which it is growing, showing differences in soil type very clearly.

in the upper layers, and it is found that deep-rooting plants can reach the lower layers of the soil, where adequate copper supplies exist. Hence in the same soil deep-rooting plants may be quite healthy, while shallow-rooting ones, such as certain cereals, may be acutely deficient. This phenomenon is found in many parts of the world, and in South African orchards deep ploughing, with its resultant sub-soil aeration which it brings about, results in deep healthy rooting systems developing, with a frequent disappearance of trace-element deficiencies [35].

While the flora of the North German Plain is an adapted one, many plants can grow in this area. But in cases where the soil is 'poisoned' by toxic levels of elements a very much smaller number of species are adapted to these conditions. Thus, mine dumps and ore deposits have their characteristic flora, as have soils derived from such rocks as serpentines. Serpentine rocks are characterized by their high levels of nickel, chromium, and cobalt, and soils derived from them tend to be toxic for most vegetation, and have their own characteristic serpentine flora. As the aerial photograph in Fig. 67 shows, this adapted flora is strictly confined to serpentine soils, and there is an abrupt change in vegetation as the soil type changes [159].

This association of certain vegetation types with specific soil conditions leads naturally to the search for *indicator plants*. In the case of prospectors, indicators for ores are sought, while in agriculture reliable indicators of soil conditions are of great practical interest, because it is difficult to assess the nutritional status of a soil by chemical means.

Indicator plants of deficiency and excess

As the mineral requirements of plants are so very varied, it is clear that certain varieties or species will have a more marked requirement

TABLE 35

Indicator plants—of soil nutritional status. From WALLACE [319]

DEFICIENT ELEMENT	PLANT
Boron	Apple
Iron	Cauliflower, broccoli, marrow-stem kale, cabbage, apple, pear, plum and raspberry
Manganese	Sugar beet, mangold, globe beet, apple, sweet cherry and raspberry
Molybdenum	Cauliflower
Zinc	Orange and apple

These plants have a marked requirement for certain of the nutrient elements, and when these are low in the soil, or not readily available to the plant, they show characteristic and readily identifiable deficiency symptoms. As a result, they can be used to *indicate* the nutritional status of the soil.

for a certain trace element than most. If its requirements are not met on a certain soil it will show deficiency symptoms before the less-demanding vegetation, and hence it can be used to *indicate* the nutritional status of the soil. Thus, cauliflower is a very good indicator of low molybdenum in soils, and if the supplies of this element are inadequate 'whip-tail' (Fig. 8, page 38) is developed and can be easily observed. Similarly, citrus is a valuable indicator of zinc status. A few more examples are given in Table 35.

But the term 'indicator plant' is also commonly applied to plants which indicate the presence of certain elements, both nutritional and non-nutritional, in the soil and are used by prospectors in their search for ores. In Europe a calamine flora exists. The calamine violet *Viola calaminaria* Lej. is almost restricted to these zinc carbonate rich soils, as are *Thlaspi calaminae* Lej., *T. cepaeafolium* Koch, and *Viola lutea* Sur [91]. These plants are thus indicators for zinc, and they are of great assistance to prospectors. Other indicators are *Amorpha canescens* Nutt., which indicates lead deposits in the U.S.A., while in Brazil *Cercopia laevivirens* Hub. is of value in gold prospecting [91].

The fact that certain plants are associated with ore deposits is of considerable importance in scientific prospecting, and has led to the development of a special branch of science known as *biogeochemical* prospecting. This subject concerns itself, among other things, with

TABLE 36

Indicator plants for ore deposits

These plants indicate the presence of certain elements, ores, or rock types. They occur primarily or exclusively on these sites. For instance, the Serpentine flora are found on soils that other plants cannot colonize due to the presence of high level of nickel, chromium, or cobalt salts. Many of these indicator plants are also accumulator plants of Table 37.

ELEMENT OR ROCK FORMATION	PLANT	AREA	COMMENT	REFER- ENCE
Copper	<i>Caryophyllaceae</i>		Many members of this family indicate copper	[50]
	<i>Arenaria verna</i>	Australia	Moss plants. Prefer copper-rich soils but will grow on iron and manganese rich ones	[50]
	<i>Mieliohofera nitida</i> }			[50]
	<i>Scopelophila ligula</i> }			
	<i>Lychnis alpina</i>	Norway		[50]
	<i>Lychnis dioica</i>			[50]
	<i>Silene</i> spp.	U.S.A.		[50]
	<i>Polycarpea spirostylis</i>	Australia		[91]
	F. M. Muell.			
	<i>Alsine verna</i> L.	Germany	Indicators of copper and zinc	[91]
	<i>Armeria vulgaris</i>	Germany		[91]
	<i>Viscaria alpina</i> (L) G. Don	Norway	Indicate copper-rich and Serpentine soils	[159]
	<i>Melandrium rubrum</i>	Norway	Copper-poisoned soils	[159]
	<i>M. dioicum</i> (L) Schinz and Thell			[313]

TABLE 36—continued

ELEMENT OR ROCK FORMATION	PLANT	AREA	COMMENT	REFER- ENCE
Gold	<i>Cercropia laetevirens</i> Hub.	Brazil		[91]
	<i>C. palmate</i> Willd.			[91]
	<i>C. tyratiloba</i>			[91]
	<i>Alpina speciosa</i> Schum.			[91]
	<i>Typha latifolia</i> L.			[91]
	<i>T. domingensis</i> Kunth.			[91]
Iron	<i>Epidendrum o'breinianum</i>	Venezuela		[50]
Iron pyrite and arsenopyrite	<i>Calamagrostis</i> sp.	Venezuela	Tree 20-50 ft. high. Can be identified on aerial photographs	[51]
Lead	<i>Tussilago farfara</i>	Germany		[50]
Lead sulphide	<i>Amorpha canescens</i> Nutt.	U.S.A.		[91]
Manganese	<i>Digitalis purpurea</i>	Switzerland and Germany		[262]
Mercury	<i>Arenaria setacea</i>	Spain		[91]
Selenium	<i>Astragalus pectinatus</i>	U.S.S.R. (?) and U.S.A.		[311]
	<i>Alpispappus Fermontii</i>	U.S.S.R. (?) and U.S.A.		[311]
				[311]
Silver	<i>Erigonum ovalifolium</i>	U.S.A.		[91]
Tin	<i>Sempervivum soboliferum</i> Sims.	Germany	Tin-mine dumps	[91]
	<i>Trientalis europaea</i> L.	Germany	Tin-mine dumps	[91]
	<i>Pluchea quiloc</i>	Brazil		[91]
Zinc	<i>Thlaspi alpestre</i>	Germany	Members of the Calamine Flora, characteristic of Calamine (ZnCO ₃) districts in Europe	[91]
	<i>Thlaspi</i> spp.	Austria, etc.		[91]
	<i>Viola tricolor</i>	Germany		[91]
	<i>Viola lutea</i> Sur.	Germany		[91]
	<i>Ruta graveolens</i> L.	Brazil		[91]
	<i>R. latifolia</i> Mart.	Brazil		[91]
	<i>Matricaria americana</i> L.	Brazil		[91]
	<i>Senecio brasiliensis</i> Less.	Brazil		[91]
	<i>Papulus deltoides</i>	U.S.A.		[91]
	<i>Ambrosia elatior</i>	U.S.A.		[91]
	<i>Ambrosia trifida</i>	U.S.A.		[91]
	<i>Viola maculata</i>	Belgium	Indicates zinc silicate	[91]

Certain mineralized areas have a characteristic flora associated with them, such as the Calamine Flora mentioned above. A Serpentine flora has been recognized for a long time, but its components vary, depending on how much chromium, nickel, or cobalt are present in this rock and the part of the world in which they are found. A lithium flora, in which many plants accumulate lithium, as well as a selenium flora, are recognized, but many of the plants found in these regions are accumulator plants rather than real indicator species.

indicator plants, and as a glance at Table 36 will show, quite a number of these are already known.

Accumulator plants

Certain plants, including some of these ore indicators, possess the remarkable ability to accumulate extremely large amounts of certain elements without suffering any ill effects. *Thlaspi calaminare* L. has been found with 20 per cent zinc oxide in its ash. This level of zinc would normally be lethal to plants.

In prospecting, this ability to concentrate elements which are present in very small quantities in the soil, and so make chemical analysis easier, is exploited. In Canada leaves and twigs of relatively ordinary vegetation are used to identify ore deposits. Balsam Fir (*Abies balsamea*) and Blueberries (*Vaccinium ovalifolium*), which contain significantly higher levels of copper and zinc when growing over ore deposits, are used for finding and mapping these [321]. The level of these metals is not high enough to warrant classifying these plants as accumulators.

True, accumulator plants can be a source of serious trouble. *Astragalus* spp. and *Oenopsis* spp. accumulate so much selenium from the soil that they poison stock grazing on pastures in which they are growing. They are primarily responsible for the 'alkali' staggers (see page 145) or selenium toxicity in cattle in the drier mid-west of the U.S.A., where the soils contain selenium. The seeds of *Astragalus racemosus* can contain over 2,000 p.p.m. selenium, while many of the plants contain so much of this element that they give off a garlic-like odour, which is characteristic of certain selenium compounds.

The reason why plants act as accumulators is not known. There appears to be a genetic factor involved, as closely related species often share this ability. Certainly soil factors can regulate the absolute amount of an element absorbed, as they do in most cases of mineral absorption, but the physiological processes involved in absorption and in resisting the toxic levels of these elements have not been investigated. This ability is not confined to plants. Certain blue oysters obtain their colour from accumulated copper, an element that is toxic at very low levels to most marine forms of life.

It is equally difficult to see what advantage this ability to accumulate certain elements is to most plants. While it may have a survival value for plants growing on ore deposits, its benefits to vegetation growing on fairly normal soils are hard to appreciate. That accumulators of many sorts exist, however, is clearly established, and some of the known species are shown in Table 37.

Succession

Successions of communities which follow each other until a stable vegetation type is established are of great ecological interest, but the part played by trace elements in such successions is not clear. In Australia quite stable clover pastures have been established on acid soils, either by liming heavily or by the application of a light dressing of lime with a little molybdenum (molybdenum is the only trace element that becomes more available under alkaline conditions, and

TABLE 37
Accumulator plants

ELEMENT	PLANT	COMMENT	REFERENCE
Aluminium	<i>Cardwellia sublimis</i> F. Muell		[323]
	<i>Orites excelsa</i> R. Br.		[323]
	<i>Marrattiales</i>	Many species of this fern	[323]
	<i>Camellia japonica</i> L. var.		
	<i>hortensis</i> Makino		[141]
	<i>Melastoma malabathricum</i> L.		[141]
	<i>Miconia androsaemifolia</i> Gris.		[141]
	<i>Lycopodium sabinaefolium</i> Willd.		[141]
Boron	<i>Polymnia</i> spp.		[157]
Barium	<i>Astragalus</i> spp.	Many Ba rich soils are sterile	[311]
Copper	<i>Polycarpaea spirostylis</i> F. M. Muell		[91]
Fluorine	<i>Dichapetalum cymosum</i>		[311]
	<i>Camellia sinensis</i> (L.) Kuntze	Tea	[311]
Lithium	<i>Thalictrum</i> spp.	Also other species of Ranunculaceae	[311]
	<i>Lycium</i> spp.	Also other species of Solanaceae	[311]
Manganese	<i>Trapa natans</i>		[311]
Nickel	<i>Pancheria glabrosa</i>		[159]
	<i>Betula alba</i>		[159]
	<i>Alyssum bertolonii</i>	Very high concs. up to 15,000 p.p.m.	[159]
	<i>Pulsatilla patens</i>		[157]
	<i>Linosyris villosa</i>		[157]
Rare earths	<i>Carya</i> sp.	Hickory tree	[311]
Selenium	<i>Alpipappus Fermontii</i>		[311]
	<i>Astragalus pectinatus</i>		[311]
	<i>Astragalus racemosus</i>	Can accumulate without injury 15,000 p.p.m.	[303]
	<i>A. patersonii</i>		[303]
	<i>Astragalus</i> spp.	Legume	[311]
	<i>Stanleya</i> spp.	Crucifer	[303]
	<i>Xylorhiza</i> spp.	Composite	[303]
	<i>Oonopsis</i> spp.	Composite	[303]
	<i>Sium cicutaefolium</i>		[306]

TABLE 37—continued

ELEMENT	PLANT	COMMENT	REFERENCE
Uranium	<i>Astragalus</i> sp.		[311]
Vanadium	<i>Amanita muscaria</i>	Fly Agaric	[311]
Zinc	<i>Armeria halleri</i>		[311]
	<i>Alsine verna</i>		[311]
	<i>Thlaspi alpestre</i>	Formerly <i>T. calaminare</i>	[311]
	<i>Viola tricolor</i>	Formerly <i>V. calaminare</i>	[311]

Certain plants can concentrate and accumulate high levels of elements from the soil with no apparent harm to themselves. In the case of aluminium, selenium, or any other non-essential element, it is possible that the accumulated element may play a nutritional role.

hence liming makes molybdenum plant available). These pastures, which need help to become established, are permanent when proper growth has occurred [7].

The part that liming and fertilizer techniques play in altering successions is not clear for many communities, but that their influence must be large will be seen in the chapter devoted to fertilizers.

Changing environments

In many parts of the world the climax vegetation has completely altered within the last century, and the character of the soil and of the plant communities has altered within living memory. If an area such as the Cape Peninsula, South Africa, is taken as an example, regular burnings have altered the environment of the local plants considerably. Larger shade plants have long since vanished in most areas, while the shorter herbage which mulched the soil and protected it from excessive heating by the sun and heavy leeching by rain has thinned out considerably. The soil is now so impoverished that the indigenous flora is no longer adapted to these new conditions, and in some parts shows severe deficiencies of trace elements, as is clear from Fig. 68. These plants are being replaced by alien vegetation more adapted to these conditions [266].

From the matter discussed, it is obvious that the trace elements have a number of important ecological facets, but unfortunately these have not been seriously studied. Even more unfortunate is the fact that very little is known about aspects of animal ecology related to trace elements. Certainly rabbits and other vermin show a preference for trace-element fertilized fields, and cattle and other animals will travel long distances to satisfy their requirements for iron and other trace elements. And in parts of the world where rivers of almost



FIG. 68

SCHÜTTE [266]

Acute deficiency in naturally occurring vegetation

Undisturbed natural vegetation may show symptoms of micro-nutrient deficiencies, but this is much rarer than in cultivated plants, as natural vegetation is usually adapted to its environment.

This photograph shows the chlorosis in *Rhus tomentosa*, growing on a hillside in the Cape Peninsula, South Africa. It is a complex deficiency, being mainly a severe manganese deficiency, with lesser deficiencies of copper and magnesium as well. Erosion is severe in this area.

pure water, which drain very leached soils, are found, their fauna is strictly limited. Are trace-element deficiencies also involved? Who knows? It is certain that many problems must be studied in some detail before it is possible even to speculate about the significance of trace elements in animal ecology.

XI | The treatment of trace-element imbalances

Full *balanced* nutrition is essential for optimal growth and development. This book has stressed the deleterious consequences of disequilibria of trace-element nutrients, be they due to deficiencies or excesses. It is clear that some effort must be made to readjust the mineral balance and to combat these conditions, be they goitre or anaemia in man and animals, or fruit production in plants, for they can cause great loss of health, as well as of crops.

However, before any prophylactic or therapeutic measures can be undertaken it is necessary to establish the nature of the condition that needs treatment and its causes. It is only when these have been established that effective measures can be introduced to deal with the condition. And of course, it is only possible to deal with the condition satisfactorily if techniques are available to do so. But the first problem undoubtedly is to establish the presence of a nutritional disturbance.

Methods for determining deficiencies or excesses of trace elements in plants

(i) *Visual symptoms*. Severely deficient plants have a characteristic clinical appearance, and many symptoms of both deficiencies and excesses in crop plants are known and recorded [319]. These symptoms can be of great value in preliminary investigations and, used by an expert, can yield much useful information.

From the practical agricultural point of view, visual symptoms are of limited value because they only appear when the deficiency is severe, and are of no value in establishing latent deficiencies. Also, it must be appreciated that simple deficiencies of only one element are rare in nature. It is more usual to find multiple deficiencies, and these alter the visual symptoms very considerably, so that it is very difficult to determine the cause of the abnormal conditions with any certainty.

Used in conjunction with other methods, the study of visual symptoms is of value in establishing the nutritional disturbances. On its own, it is of limited use.

(ii) *Soil Analysis*. As imbalanced nutrition is usually the result of disequilibria in the soil salts, it would seem that chemical analysis of the soil might give the desired insight into the mineral requirements

of crops. But unfortunately no satisfactory method for analysing the *plant available* nutrients in the soil exists. Certainly there are numerous chemical techniques for measuring mineral nutrients in soils, but they are of limited value, because their results are hard to interpret. In countries with old well-established farms soil analysis can yield some information, in cases where a long background of empirical experience exists, into which the analytical data can be fitted and interpreted. But in newly developed areas, which are very extensive in America, Africa, and other parts of the world, this background

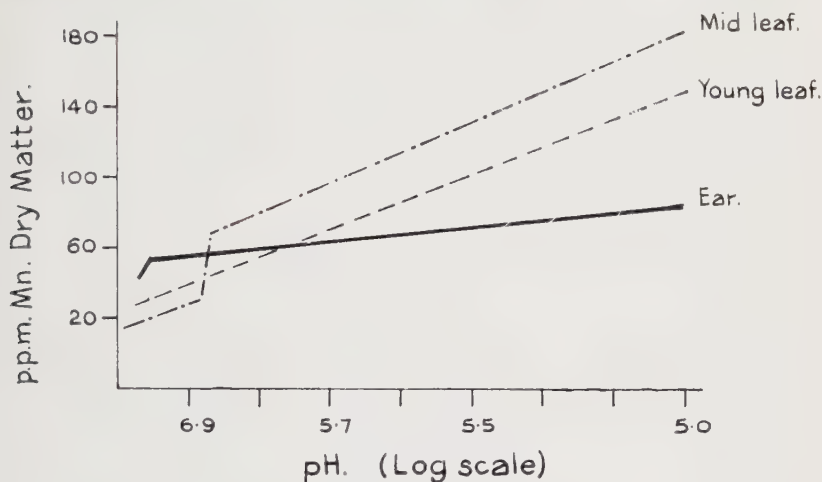


FIG. 69

FINCK [101]

The manganese content of various parts of the oat plant

It is clear that the manganese content of the plant is not uniform, and that the concentration of this element in grain, young leaves, and older leaves of the same plant can be quite distinct. The pH can influence the manganese content very considerably: leaf levels are highest under acid conditions.

does not exist, and the significance of soil analysis is hard to determine.

Chemical analysis of soils can determine absolute, i.e. total, levels of various nutrient elements accurately. *This method can show up absolute deficiencies or excesses.* It is not a good method for determining the amount of material that is *plant available* in a soil. Plant availability depends on far more than mere presence in the soil. Iron chlorosis is frequently encountered on red laterite soils, which are very rich in total iron, but may be poor in available iron. From the chemical point of view, it is almost impossible to determine what is plant available, as availability varies, first, with the species of

plant, then with the physical nature of the soil, climate, microbial activity of the soil, etc.

(iii) *Leaf analysis.* This is a very useful and widely used technique, in which leaf samples are analysed. As the mineral content of the leaf is a very good index of the available elements in the soil, and also of the plant's requirements, this method is used extensively for routine

TABLE 38

The spectrographically determined levels of iron and manganese, and their relationship to the presence and absence of visual deficiency symptoms in peach leaves, in Cape orchards. From BEYER [38]

NUMBER OF ORCHARDS			NUMBER OF ORCHARDS		
ELEMENT	NO		ELEMENT	NO	
Mn, P. P. M.	SYMP- TOMS	SYMP- TOMS	IRON, P. P. M.	SYMP- TOMS	SYMP- TOMS
10	2	(27)	40	0	(2)
20	5	(9)	60	3	(3)
30	15	(5)	80	22	(3)
40	26	(1)	100	25	(2)
60	19	(0)	120	22	(0)
100	10	(0)	140	10	(0)
150	9	(0)	160	1	(0)
200	2	(0)	180	2	(0)
250	2	(0)	200	1	(0)
300	2	(0)	220	1	(0)
350	1	(0)	240	1	(0)
400	0	(1)			
<i>Iron</i>	Critical Level		60 240 p.p.m. (in this area)		
<i>Manganese</i>	Critical Level		30-140 p.p.m. (in this area)		
	Toxic Level		400 p.p.m. (in this area)		

It is clear from the above table that deficiency symptoms are not present in *all* orchards where the manganese content is below the critical level. Again quite a large number of orchards where the level is above the minimum critical one do show deficiency symptoms. In fact, in one orchard where toxic concentrations of this element were present deficiency symptoms were observed.

The same is true for iron, and deficiencies were frequently observed above the minimum critical level.

Clearly the level of these elements and the occurrence of visual deficiency symptoms are not directly related, although they correspond approximately in most cases.

analysis. Most of the elements are estimated by sensitive and rapid spectrographic analyses of leaf ash, and its sensitivity and speed have made it very popular.

It is of the utmost importance that rigorously standardized sampling techniques be used in this method, as not only its age, but also its

position on the parent, influence the mineral content of a leaf, as can be seen very clearly in Fig. 69.

While the leaf content is normally a good index of the plant's mineral requirements, it must not be forgotten that plants can and do absorb high levels of nutrients that are not used in its metabolism. High levels of iron or manganese are known to occur in the leaves of plants suffering from deficiencies of these elements. That these are real deficiencies of available iron or manganese is shown by their beneficial response to nutrient sprays. This state of affairs is clearly shown in Table 38, which is based upon analytic data obtained in a fruit-growing region in South Africa.

Other factors besides age influence the mineral content of leaves, and the requirements of plants. Some of these, like light intensity, drought, temperatures, have been discussed in Chapter X.

It is clear that leaf-analysis techniques are both rapid and convenient, but again they must be interpreted in an empirical background of experience. As they reveal the composition of the plant, they are of more value than soil analyses, although they do not show directly the plant's physiological status or requirements.

Leaf injection techniques

Roach [237, 240, 241] developed a technique that is simple, elegant, and informative. It is misleadingly termed an injection technique. Apart from its simplicity, this method is useful because it measures biological responses, and obviates many of the real difficulties encountered in chemical determinations as a result of differences in plant availability of the nutrients.

In this technique a small incision is made in the leaf lamina, and a small container filled with a very dilute solution of the element being studied is attached to this cut by a wick from the container. The manner of using this is shown in Fig. 70. The solution flows up this wick by capillarity and becomes available to the leaf tissue. If the element is deficient, then within two to three weeks growth responses are visible, and the inter-veinal region becomes dark green in colour. The rest of the leaf functions as an untreated control.

This method is rapid and cheap. It has the advantages of being a biological method, and finally it is carried out in the field and enables the operator to observe if environmental factors, such as drainage, may be inducing malnutrition.

Fertilizer trials

Perhaps the most informative method for studying plant nutrient requirements in the field is by means of fertilizer trials. Their great drawback is that they are difficult to lay out, and may take a number of years to complete. Hence they are very expensive.

For a proper fertilizer trial the plots must be large enough to give satisfactory results, and must be properly randomized to prevent any uncontrolled local factor upsetting the experiment. *Ideally, all the macro- and micro-elements should be included*, at various levels, so that element interactions and induced deficiencies may be observed. As all elements interact to give rise to the balanced nutrition necessary for well-being, such an experiment is an ideal one, but in practice,

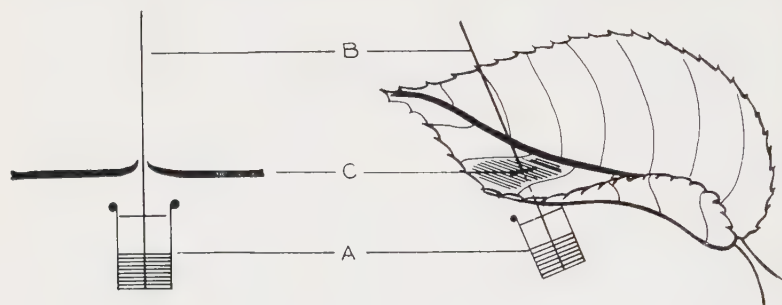


FIG. 70

ROACH [240]

The Roach leaf injection technique

- A. Plastic container of solution with wick attached.
- B. Paper wick, hardened to give mechanical strength.
- C. Leaf, with incision to allow the entry of the wick.

This diagram shows the simplicity of the equipment required for this technique. The liquid is contained in a container (A) which is cut from a polyvinyl chloride drinking straw and sealed at the bottom. The strip of filter paper which is stuck to the bottom functions as a wick (B) and can be passed through an incision in the leaf (C). The upper part of the wick is painted with cellulose acetate to harden it, so that it can support the container without difficulty. The liquid travels up the wick and into the leaf, where it is absorbed.

The diagram shows the nature of the response when a deficient nutrient is supplied. The part of the leaf between the veins turns a healthy dark green, while the rest of the leaf remains pale, and so acts as its own control. The healthy growth that ensues in the dark-green region may result in slight corrugations being formed, as the rest of the leaf does not grow at the same rate.

A good response to an element indicates that it is deficient.

they are far too complex to be undertaken, except on very rare occasions.

Smaller-scale experiments only attempting to investigate part of this ideal are often conducted, and do, in fact, yield useful practical information. The least valuable of these trials are those in which only one nutrient is added at several levels and no interactions with any other elements are studied. The drawbacks to this type of investigation will be quite clear from the discussions in the next chapter, where fertilizer-induced deficiencies are discussed.

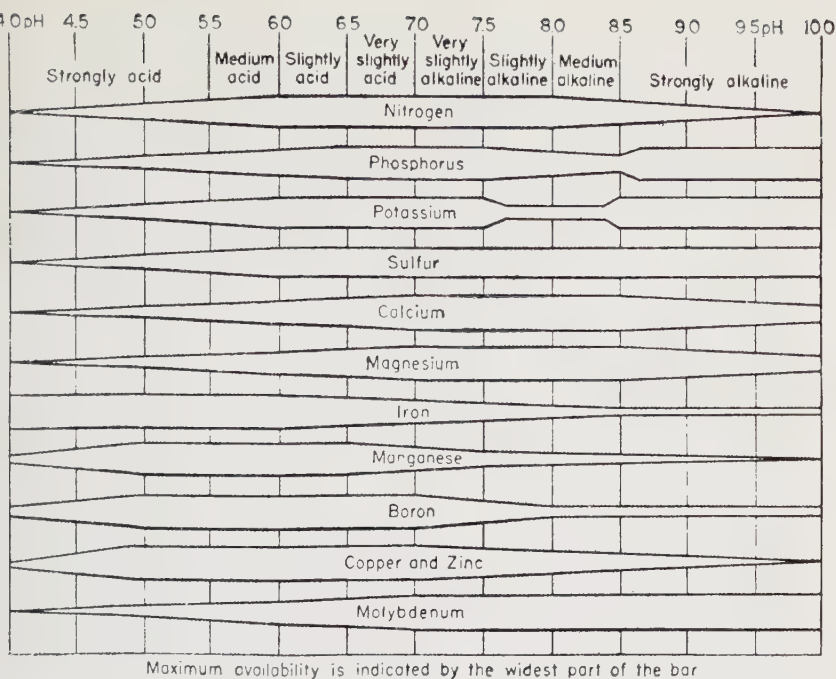


FIG. 71

AFTER TRUOG [304A]

The influence of pH on the availability of plant nutrients

The simple addition of nutrients to a soil does not mean that they are automatically available to the plant and if the soil is alkaline, then many are converted into forms which are not available to the plant. Others are not readily available in acid soils. The reactions of both micro- and macro-elements are shown.

The diagram shows the influence of pH* on availability in determined average conditions. Many good agricultural soils are slightly acid, and overliming results in the soils becoming alkaline. This may, and frequently does, induce deficiencies of micro-nutrients such as manganese, which are not readily available under alkaline conditions.

It must not be thought that good fertilizer experiments can give all the desired answers, as changes in climate and other environmental factors may alter the plant requirements very considerably, and old experimental data may no longer be applicable.

For instance, the acidity or alkalinity of a soil is something that alters the availability of the nutrients in the soil. Under alkaline conditions the commoner trace elements (excluding molybdenum)

* The pH is a measure of alkalinity and acidity. It measures from pH 1 (very acid) to pH 14 (very alkaline). pH 7 is neutral, being neither acid nor alkaline.

are less available to plants. Thus, agricultural techniques which make the soil alkaline may *induce* deficiencies. In practice, *over-liming* causes much trouble, although liming is essential to good agriculture in many areas, as many crops thrive on neutral or slightly alkaline soils.

Sulphuring, or the use of fertilizers like ammonium sulphate, tend to turn soils acid. This again alters the balance of the available nutrients in the soil, and in some areas may induce molybdenum deficiency. In fact, soil reaction plays a great part in determining the nutritional status of the soil, as must be clear from charts such as the one shown in Fig. 71.

It must be clear that it is not easy to develop a good fertilizer mixture, and that it is almost impossible to develop one that is optimal under a wide range of agricultural conditions. There are no 'cure-for-alls' in either medicine or agriculture.

Corrective treatment

The widespread existence of trace-element deficiencies calls for effective practical methods to overcome these imbalances. The fact that the modern agricultural techniques result in progressively heavier crops being removed from the soil means that the amount of trace-element material removed is greater than formerly. Table 39 gives

TABLE 39
Amounts of trace elements removed by cropping from the soil. From
GERICKE [114]

YIELDS, METRIC TONS/HA.*	QUANTITY REMOVED, G.			
	Mn	Cu	B	Co
3 Cereal grain + 6.5 Straw	330	32	12	3
3.7 Peas and Beans + 4.5 Straw	100	50	55	28
25 Potatoes	45	42	63	6
40 Sugar beet + 26.5 Tops	400	100	300	11
60 Fodder beet + 15.0 Tops	300	80	200	9
4 Clover Hay	150	35	40	24 (?)

* 1 hectare = 2.47 acres.

The yields quoted here are very average ones. Good crops can be considerably heavier.

some indication of the rate at which cropping removes trace elements from the soil. As the rate of soil breakdown has not increased appreciably, this heavy cropping results in a drain on the available reserves of the soil, and deficiencies are becoming more common, especially on intensively cultivated soils, even where these have ample supplies of total but unavailable reserves. If the normal processes of soil breakdown cannot replace material lost by cropping the elements must be returned in other ways if fertility is to be maintained.

The macro elements, nitrogen, phosphorus, and potassium are normally returned to the soil in the form of artificial fertilizers. Manures are good material for fertilizing soils, but they are very bulky, and they are not available in sufficient quantity to meet the requirements of contemporary agriculture. So in spite of possessing certain advantages over artificial fertilizers, they no longer play the important role that they did formerly. Nor are they a very good source of trace elements, and Table 40 gives some average contents and compares them with other fertilizers.

TABLE 40

The approximate trace-element content of some manures and fertilizers in g./100 kg. From KICK [151]

FERTILIZER OR MANURE	COPPER	MAN- GANESE	ZINC	MOLYB- DENUM	BORON
*Stable manure	0.2-0.7	3-4	1.5-2.0	0.05	0.3-0.6
*Liquid manure	0.04	0.1	—	Trace	0.2-0.4
*City compost	13-20	~50	30-200	1.0	5
Superphosphate	2-4	2-8	~40	0.1	0.8-1
Nitrochalk	10-15	10-12	Trace	Trace	—

The organic manures (*) are not an unduly rich source of trace elements, except that in this case the zinc content of the city compost is high. As these organic materials are very bulky, and it is seldom possible to give optimal applications of these to fields, they must be regarded as relatively poor sources of trace elements, and are not always likely to make good soil deficits. However organic manures increase the activity of soil micro-organisms which transform *total* mineral elements to *available* elements.

Although a number of fertilizer mixtures contain some of the trace elements in them, it is more usual to supply the trace elements separately.

The trace elements can be supplied as soil additives, and boron, for instance, is widely supplied as borate to the soil, the rate of application depending on the crop, the nature of the soil, and the climate. But the application of trace elements is not always so easy, as elements such as copper, manganese, or iron get 'fixed' very rapidly by the soil. This means that they are rapidly turned into unavailable forms in the soil. When this is the case soil applications may not be the most economical way of combating deficiencies.

Most modern crops are sprayed as a matter of course as a precaution against infection. This is particularly true of most fruit and many vegetable crops. In such cases the trace elements can be supplied as foliar sprays, which is a very efficient way of supplying these nutrients. As they can be mixed with the fungicidal sprays, the nutrients can be applied two to three times in a season at a relatively slight expense. Roach [240] gives some actual costs, which are shown in Table 41, which show very clearly the benefits obtained from spraying.

As many of the deficiencies in the field are complex deficiencies of two or more elements, spraying is one of the simplest methods of treating these conditions when intensive agriculture is being practised.

But other techniques for combating these imbalances are also used. As well as applying salts directly to the soil, where they are 'fixed' fairly rapidly, it is also possible to apply *frit*. Frit is a glass which

TABLE 41

The effect of spraying with manganese sulphate on the yield of various crops in a manganese-deficient area and their increase in value and the cost of the treatment. From ROACH [240]

CROP	YIELD PER ACRE		IN- CREASED YIELD, CWT.	IN- CREASED VALUE, £	COST OF SPRAY- ING, £
	UN- SPRAYED, CWT.	SPRAYED, CWT.			
Wheat	18.5	35.5	17	12	3
Barley	22	36.5	14.5	18	2
Peas	9	20	11	26	1
	TONS	TONS	TONS		
Parsnips	16.5	21	4.5	80	1.5

It can be seen that for every £1 expended on spraying in this manganese-deficient area the farmer receives back £4 in extra wheat, £9 in extra barley, £26 in extra peas, and £53 in extra parsnips. The treatment pays handsomely for the expenses incurred.

has large amounts of trace elements in it. It is ground to a very coarse powder and applied to the soil. In the soil it breaks down slowly, liberating the micro nutrients it contains, and continuously supplying these nutrients to the soil for years at a time.

As well as these main methods of direct salt applications to the soil, foliar sprays and the use of frit, more specialized methods, such as inserting nutrient pills into holes bored in stems of trees, or burying bags of salts near rooting systems, are used to a limited extent in practical farming.

Chelates

The fact that the trace elements exert most, if not all, their effects while they are attached to organic molecules of some complexity has been known for a long time, but it is only within the last twenty years that much insight has been gained into the nature of these complexes, which are of much interest to biologists. Of these complexes, the *chelates*, with their very special properties, are of particular interest.

A chelate is a compound formed between a metallic ion and an organic molecule having two neighbouring groups capable of simultaneously combining with the metal to form a ring structure. The chelate resembles a claw, with the metal ion clutched between the

pincers of the organic molecule, and so the term chelate is derived from the Greek word for a claw.

Chelates have very high stability, and as a result, the metal in them is no longer available for many of its usual reactions, and many of the biological, chemical, and physical properties of the metal are changed. Chenoweth's [65] review is a very fine introduction to the biological aspects of these substances, while Wallace's [318] book deals with their effect in plant nutrition. A more general account of these complexes, mainly from a chemical viewpoint, is that of Smith [280].

While not very much information is available about naturally occurring chelating substances within tissues, it appears that metals are frequently translocated as chelates within tissues [301], and it is possible that boron chelates may play an important part in the translocation of sugar within plants [110]. However, it is in synthetic chelating agents, such as *ethylenediaminetetracetic acid* (EDTA), that most interest is centred.

EDTA is probably the most potent chelating agent known, and the chelates that it forms with heavy metals are very stable. In mammals it is very effective for detoxicating poisonous metals such as lead, which are then excreted as non-toxic chelates. The toxicity of this compound is so low that lead EDTA is used clinically as an X-ray contrast material [65]. In practical agriculture and also in plant research one of the most valuable uses of EDTA is to make iron readily available. Iron is an element that is rapidly made unavailable under natural conditions, and tissues often experience great difficulty in obtaining adequate supplies, even in the presence of large quantities of total iron. Chelating agents enable plants to tap soil reserves of iron efficiently, and in culture solutions iron chelates maintain the level of available iron in the solutions [49]. Chelates of nutrient metals can also be sprayed on to vegetation.

Methods for determining trace-element imbalances in animals

In general, visual symptoms are of greater importance in animal studies than in plants. The feeding of trace-element-fortified supplements can result in the cure of mild deficiencies before they are even adequately identified. But such a state of affairs is obviously unsatisfactory, as live weight, milk yield, fertility, and many other desirable characteristics may be detrimentally influenced before visual clinical symptoms can be identified.

Fodder analysis

A knowledge of the mineral composition of animal fodder gives valuable insight into the nutritional status of the animal. This is

particularly true of herbivores, which do, in fact, constitute the bulk of the agricultural animals involved in trace-element disequilibria.

Herbage analyses from Germany would indicate that low levels of such elements as manganese and copper are so widespread as to constitute a serious agricultural problem. In Table 42 the manganese

TABLE 42
The manganese content of German hays. From GERICKE [114]

MG. Mn/KG.	% FREQUENCY		
	1ST CUT	2ND CUT	1ST AND 2ND CUT
<50	27.9	20.7	24.3
51-100	31.6	30.7	31.2
101-150	17.6	19.4	18.5
151-200	9.0	8.2	8.6
201-250	4.2	8.0	6.1
251-500	7.0	9.1	8.0
500	2.7	3.9	3.3

50 mg. Mn/kg. is considered marginal for hay in Germany, and animals fed on hay containing less tend to develop manganese deficiency.

It is important to observe that nearly 30 per cent of the first cut hay and 20 per cent of the second cut is below this critical level, while high levels are rare. Sub-marginal levels of manganese are obviously very widespread in Germany.

contents of German hays are given, and one-quarter of the samples are below the critical level required by cattle. The copper content of hays gives as much cause for concern, Table 43 [208], where over 50

TABLE 43
The copper content of hays in the Mecklenburg area. From NEHRING AND BORCHMANN [208]

YEAR	NUMBER OF ANALYSES	POOR QUALITY, <5 MG. Cu/KG.	PASSABLE QUALITY, 5-7.5 MG. Cu/KG.	GOOD QUALITY, 7.5-15 MG. Cu/KG.	VERY GOOD, >15 MG. Cu/KG.
1959	476	57%	38%	5%	—
1960	345	64%	36%	—	—

If the critical level for copper is 5 mg. Cu/kg., then over half these hays are deficient in copper. It is more usual to regard 8 mg. Cu/kg. as the critical level in fodder for cattle, in which case the bulk of these hays must be regarded as unsatisfactory.

The authors stress that within the last few years the number of hays deficient in copper has been increasing steadily.

per cent of the samples must be considered deficient, and there is analytical evidence to show that this deficiency is spreading.

These deficiencies in the fodder can be made good by fertilizing the

lands with copper or with manganese. Even such elements as cobalt, which do not appear to be essential to plants, but are to animals, can be enriched in the vegetation by applying their salts to the soil. Table 44 shows that soil applications of cobalt chloride do, in fact, result in marked increases of cobalt in vegetation, *even when applied with lime*, which antagonizes its uptake.

TABLE 44

The influence of soil applications of cobalt chloride and of chalk on the cobalt content of plants. From KÜHN [161]

PLANT	CHALK	APPLICATION OF CoCl_2 , KG./HA.		
	APPLICATION	0	10	20
Cress	$1 \times \text{CaCO}_3$	0.19	0.71	1.44
	$2 \times \text{CaCO}_3$	0.19	0.41	0.68
	$3 \times \text{CaCO}_3$	0.11	0.37	0.83
Carrot roots	$1 \times \text{CaCO}_3$	0.17	0.38	0.49
	$2 \times \text{CaCO}_3$	0.14	0.17	0.26
	$3 \times \text{CaCO}_3$	0.12	0.16	0.23

Cobalt content is expressed as Co p.p.m. dry matter. Soil applications of cobalt result in enhanced cobalt contents of the plants, even when added with high levels of chalk.

In many parts of the world the soils are not productive enough to permit very intensive grazing, and extensive farming is practised. As a result, it is frequently not economic to try to overcome these problems by treating the soil. Under these circumstances it may be possible to feed the animals concentrates containing the desired elements, or to include them in either salt licks or in the drinking water.

Indicator organs

It is very desirable to be able to establish the presence of mineral imbalances in animals before these have caused severe damage. Ideally, a simple, inexpensive yet reliable test that does not damage the animal is required. These can then be used in a similar way to leaf analyses in plants. The results obtained can be used to determine further treatment.

A very fine example of such a test is one in which analysis of the hair of cattle for manganese appears to give a reliable index of the manganese status of cattle. As the latter is directly related to fertility, this is obviously a test of real practical importance. In Table 27 (page 107) some figures are given to show the close relationship between the manganese content of hair and fertility in cows. In the Hanover area of Germany levels of below 10 p.p.m. Mn and above 20 p.p.m. indicate decreased fertility [188]. Daily oral supplements of

manganese can maintain this element at its required level. This method is obviously more practical than analyses of the ovary, an organ which also reflects manganese levels very clearly [32].

Unfortunately, hair is not a good index of copper status, as was shown in New Zealand, and both molybdenum and sulphate influence the copper content of hair [75]. Blood-serum copper measurements can be readily made, but these are not as accurate an indication of copper status as liver copper. The almost linear relationship between liver copper and copper intake is shown in Fig. 50 (page 115). New techniques enable liver biopsies to be conducted quite safely on both cows and calves, and these appear to be the most accurate way in which to gauge copper status [308].

Liver, blood, ovaries, and hair are the main tissues used in investigations of animals. From the analysis supplied, practical steps can be taken to remedy the imbalance.

Treatment of animals

Ideally, the animals should be fed on fodder containing adequate levels of the mineral nutrients. The application of fertilizers to the soil can result in the plants acquiring adequate quantities to meet the animals' needs. Or the salts of the desired elements may be incorporated in other feedstuffs. This method is not always as simple as it appears, because certain salts have a bad taste and taint food. High levels of copper sulphate in pig-feed prevented the animals feeding freely on it [27]. Many iron salts have a bad taste, and this makes oral supplementation in both animals and man somewhat difficult.

TABLE 45

Blood copper levels in calves after receiving intramuscular injections of 400 mg. anhydrous copper glycine (i.e. 120 mg. Cu) in the buttocks. From MORGAN et al. [196]

GROUP	μG. Cu/100 ML. BLOOD	
	BEFORE INJECTION	2 MONTHS AFTER 1ST INJECTION
Control	88 ± 10	46 ± 7
1 Injection	83 ± 7	73 ± 5
2 Injections	73 ± 8	81 ± 4

The injections caused the blood copper to remain at a high level. One injection gave approximately 10 per cent weight increase in the suckling and grazing period.

1-4 injections per year keep the animals thrifty.

In free grazing animals injections of copper compounds are very popular. Injections are more convenient and more efficient than other therapeutic procedures. It is of little consequence if the injection is subcutaneous or intramuscular. An injection of 120 mg. Cu as copper

glycine supplies the needs of an animal for two to three months [196]. The data in Table 45 show how such injections maintain an adequate level of copper in the blood. In Australia sheep show the same beneficial responses as cattle [100], and in Britain both respond well [2].

A number of nutrients besides copper can be injected very beneficially. These include iron, manganese, zinc, cobalt, and selenium. But it must be borne in mind that cobalt injections are of no value in conditions such as Phalaris Staggers [83], where the cobalt is apparently involved in some detoxication process in the rumen.

A very novel and effective method can be used to combat cobalt deficiency in sheep. These animals are unable to retch, and a cobalt 'bullet' is shot at a low speed into their gullet, where it remains for life, as the animal cannot get rid of it. This 'bullet' supplies the animal's needs and does not appear to cause any harm. In general, this has been found to be a very efficient method, but in parts of Scotland it is of limited value, as the bullet becomes encrusted with calcium carbonate, which prevents any cobalt dissolving and so entering the digestive system [217].

Trace-element therapy in man

Reference has already been made to the prophylactic addition of iodine to salt in an effort to combat goitre. This is a very simple measure that has very appreciable beneficial results. But the etiology of goitre is very complex, and there are other causes beside iodine deficiency, so it is not surprising that salt iodization does not cure all goitres. In fact, some of them prove very resistant to treatment.

The beneficial influence that fluorine exerts on teeth has also been discussed, and the fact that many urban centres add this element to their drinking water to prevent caries was mentioned. This is a measure that frequently meets with much opposition, as fluorine is so toxic, and many people fear cumulative effects which would be worse than caries would be.

It is truly astonishing how widespread simple iron-deficiency anaemia is. Although other anaemias involving copper, cobalt, and manganese are known, this simple iron-deficiency one is very widespread, even in the countries with a high standard of living. It is particularly common in infants who live on restricted diets, and in women because of the regular drain on iron reserves due to menstruation, gestation, and lactation. It has been estimated in the *U.S. Yearbook of Agriculture* that 40 per cent of all pregnant women in America would benefit from iron therapy [195]. These anaemias respond to oral as well as injected iron. As iron salts often have an unpleasant taste, and, moreover, are not readily absorbed in the

digestive tract, injections or the use of iron chelates are of the greatest practical importance.

As was shown in Chapter VIII, the trace elements play a very important part in all phases of blood production. Copper, manganese, and cobalt are just as essential for good and normal blood formation as iron is, while vanadium, if not essential, at least plays a very beneficial role [274, 304]. In the light of what has been discussed, it is not surprising that many tonic preparations, for patients who are run down and slightly anaemic, contain mixtures of trace elements. However, Seibold [275] points out that there is no experimental justification for this procedure in most cases, and that because of the narrow tolerance limit to some of the elements included, it may even be dangerous, as toxic quantities may easily be given over a long period. He is of the opinion that there is much abuse of trace elements in certain pharmaceutical tonic preparations. It is certainly a point that must be well considered.

The use of trace-element-containing chemicals is widespread in experimental chemotherapy, and compounds such as *pyrval* (pyridoxal phosphate and vanadium salts) have shown promise in the treatment of certain rat cancers [33], but in general too little is known in this field to allow any comments of value. Certainly the trace-element balance is of importance in disease. Schroeder [257] advocates the use of the calcium salt of the powerful chelate EDTA in the treatment of high-plasma cholesterol in man. This probably results in an altered availability of other trace elements within the patient, and as was mentioned, vanadium and other trace elements influence cholesterol synthesis very considerably [76, 78]. After oral EDTA has been administered the quantity of toxic metal excreted in the urine is greatly increased [65] but the relationship between chelates and trace elements is not clear.

There is thus enough information on hand to show the importance of the micro-elements in human therapeutics, but it is still too early to evaluate their importance, or even to indicate with confidence where important advances are likely to be made in the near future.

XII | The abuse of the major element fertilizers: some consequences

'Four fertilizer mentality'

How is it possible, when it is generally recognized that at least thirteen elements are essential for plant growth and development, that most agricultural practices still continue to base their fertilizer applications on the assumption that three, or maybe four, elements are all that is necessary in practical agriculture? This 'four fertilizer mentality' is still so widespread, and its consequences so detrimental to good agriculture, that it must be discussed in some detail.

It must be stressed that it is frequently overlooked in practice that all nutrient elements interact and influence each other to a considerable extent. In Fig. 10 (page 41) Mulder's interaction chart shows how nutrients antagonize or stimulate each other's availability in the soil. It is clear that heavy applications of one element can result in a marked depression in the availability of another, and the nutrient *balance* can be upset very readily. *The level of one nutrient in soil cannot be changed without influencing the others.*

Heavy dressings of one element may induce relative deficiencies of others, and as Liebig's Law of the Minimum points out, it is the element in the *relative minimum* that governs the yield. Overfertilization can and does decrease yield, and Voisin's Law of Maximum, which is in fact but an amplification of Liebig's contention, stresses that excesses can also limit yield.

There is a group of agriculturalists who are too conservative in their opinions regarding the need for complete and balanced fertilization. They feel that trace elements did not constitute a problem one or two generations ago, and they conclude that this talk about their importance is purely academic and of little practical significance. These people, who believe that what was good enough for their fathers is good enough for them, overlook a number of very important facts. They forget that an agricultural revolution has occurred in our lifetime. Yields have increased at a fabulous rate, and many agricultural lands are yielding up to four times what they did at the turn of the century. A good example of this is seen in maize production, where the introduction of the hybrid maize varieties increased yields

by about 50 per cent. The consequence of all this increased productivity is that the removal of the trace elements from the soil has gone on at a much greater rate than formerly, and deficiencies are frequently found on the most progressive farms. Crops remove trace elements in very appreciable amounts, as can be seen in Table 39 (page 168). Recently Braun [47] has again stressed this very real problem and its implications. The fact that modern fertilizers tend to be chemically purer than formerly (hence cheaper to transport), and so do not contribute so much in the way of 'impurities' to the soil, is also forgotten.

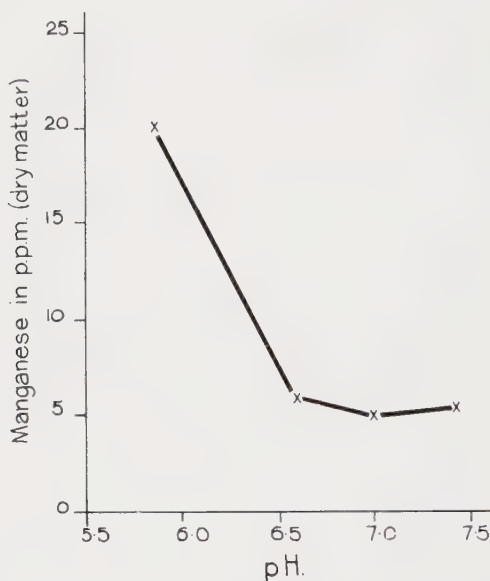


FIG. 72

AFTER HASLER [125]

The influence of soil pH on the manganese content of Fromental Grass

The marked decrease in the manganese content of Fromental Grass (*Arrhenatherum elatior*) with rising pH has been observed in other species as well. It is important to note that the manganese content has dropped by about 75 per cent within less than one pH unit. This is not regarded as a very large change in soil reaction.

New agronomic methods are also spreading rapidly. In a continent like Europe about 80 per cent of its food is still produced on its own soil. Thus, if new techniques, or their abuse, lead to marked alteration in the quality of crops this will result in very widespread social as well as purely agricultural difficulties. These are all aspects of agriculture that must be given serious consideration, and the anxiety of workers such as Voisin [315] is well founded.

Liming

'Liming is essential to maintain fertility.' This observation of Mitchell's [193] is true not only for Britain but for many parts of the world where lime shortage is one of the principal factors restricting yields. Limestone or chalk is usually added to a soil to make it less acid, and so more suitable for agriculture. In general, it is supplied as a soil conditioner, rather than as a nutrient. But because it can alter the acidity of the soil, its abuse in over-liming leads to many nutritional difficulties. The influence of soil pH on the availability of micro-nutrient elements is clearly shown in Fig. 71. Over-liming results in the soil becoming alkaline, and it is clear that iron, manganese, copper, boron, and zinc become less available as a result of

TABLE 46

The influence of liming and ammonium sulphate fertilization upon the trace-element content of barley. JUNGERMANN [145]

ELEMENT	WITHOUT $(\text{NH}_4)_2\text{SO}_4$		WITH $(\text{NH}_4)_2\text{SO}_4$	
	— LIME	+ LIME	— LIME	+ LIME
Concentrations in p.p.m.				
GRAIN				
Boron	1.3	1.1	1.2	1.1
Cobalt	0.03	0.06	0.03	0.05
Copper	5.8	6.3	4.2	5.1
Manganese	42	18	84	28
Zinc	31	35	32	38
STRAW				
Boron	26.9	8.5	25.2	8.1
Cobalt	0.29	*	0.33	*
Copper	5.9	4.2	6.2	4.5
Manganese	308	36	738	107
Zinc	132	26	87	47

* Too little to measure.

Liming results in a much decreased manganese content of both grain and straw. The influence upon other elements is not so consistent or so extensive, although the boron and zinc content of straw is considerably depressed, as is its cobalt.

The ammonium sulphate fertilization enhances the manganese content considerably, in either the absence or presence of lime. Other trace elements are also influenced, but to a lesser extent.

this. The graph in Fig. 72 shows very clearly how the manganese content of a leaf is related to the pH of its substrate and the dramatic changes that take place as a result of alterations in the pH of the soil.

That this effect of over-liming is due to an alteration in pH and not an excess of calcium ions is readily demonstrated. Wear [322] showed that applications of 2,000 lb. CaCO_3 /acre greatly reduced the

zinc content of sorghum. But the pH of the soil rose from 5.7 to 6.6. On the other hand, 2,000 lb. CaSO₄/acre increased the zinc content of the plants, *but decreased soil pH to 4.8*. So the calcium alone could not be the cause of the zinc deficiency. When other salts, such as sodium carbonate, which also turns the soil alkaline, also induce zinc deficiency, then there is little doubt that over-liming is harmful because it alters the soil pH in an unbeneficial way.

Jungermann [145], in a more detailed investigation upon the influence of liming and fertilizers upon the trace-element composition of crops, shows very clearly how extensive the alterations in mineral content can be. The figures for barley quoted in Table 46 are an example of these. The spectacular changes in manganese and in zinc must be of great importance if this material is used as animal fodder.

It must be stressed that over-liming is the bad agricultural practice, not liming. But even here, it is not always easy to generalize, for heavy liming may be very beneficial under conditions where molybdenum is in short supply. Legumes such as lucerne (alfalfa),

TABLE 47

Availability of molybdenum to lucerne as influenced by liming. From ROBINSON et al. [244]

TREATMENT	1ST HARVEST		2ND HARVEST		SEEDS	
	AVERAGE YIELD PER POT, G.	Mo CONTENT, P.P.M.	AVERAGE YIELD PER POT, G.	Mo CONTENT, P.P.M.	AVERAGE YIELD PER POT, G.	Mo CONTENT, P.P.M.
Control	3.03	1.8	13.8	1.1	1.1	2.1
Fertilizer only	7.81	2.0	59.3	0.5	3.1	2.8
Fertilizer + dolo- mitic lime	8.70	6.2	56.7	2.5	5.2	5.5
Fertilizer + dolo- mitic lime + CaCO ₃	9.04	13.1	60.7	6.7	5.9	10.0

Yields expressed as oven dry weights (80° C).

Molybdenum content determined in dried materials (105° C).

The molybdenum content of the first harvest is increased over ten-fold by liming. In crimson clover and Austrian winter peas similar responses are obtained, but heavy liming did not alter the molybdenum content of rye-grass very appreciably.

crimson clover, and Austrian winter-peas, which have an appreciable molybdenum requirement, benefit from such treatment, as it makes the molybdenum more plant available. As can be seen from Table 47, the liming not only increases the yield but also increases the molybdenum content of lucerne almost ten-fold [244].

This increased molybdenum uptake is not always as beneficial as it may appear at first sight, because if the level of this element rises too much a molybdenum-induced copper deficiency may very easily be found in grazing animals.

Nitrogen fertilizers

There is no doubt whatsoever that correct use of nitrogen-containing fertilizers is one of the really practical ways of promoting lush vegetative growth. It is equally clear that in many parts of the world greater use could be made of these compounds. But what is not so well known is that there are real dangers associated with the abuse of these fertilizers.

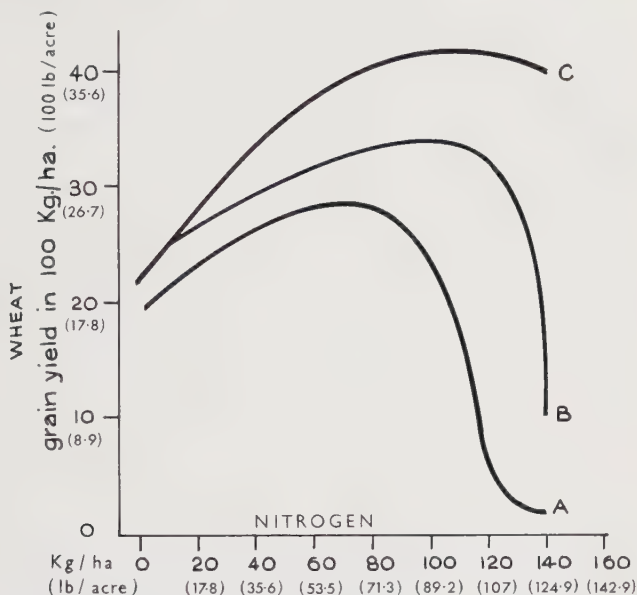


FIG. 73

MULDER [201]

The effect of different amounts of ammonium nitrate on the response of wheat to added copper

- A. No soil application of copper sulphate.
- B. 8.9 lb/acre [10 kg./ha.] copper sulphate.
- C. 178 lb/acre [200 kg./ha.] copper sulphate.

This field experiment shows how heavy soil dressings of nitrogenous fertilizers can depress grain yield in wheat. Nitrogen gives *increased* yields as long as adequate copper supplies are available. If a relative copper deficiency is induced yields drop. On the other hand, if copper is supplied, then much higher levels of nitrogen can be utilized beneficially.

As nitrogenous fertilizers bring about increases in crops, it is clear that the plant's requirements for all the other essential nutrients increases, if balanced nutrition is to be maintained. But as there is also an antagonism on occasions between these elements, this requirement can be greater than expected at first sight. Jungermann [145] found

that all his nitrogenous fertilizers, except one, decreased the copper concentration of his barley on unlimed plots.

Mulder's [201] graph (Fig. 73) shows the limiting action that lack of copper can exert on the beneficial effects of nitrogen fertilization. In a field with a relatively low copper status added nitrogen gave increased yields within limits. Any additions of more than 80 kg. nitrogen per hectare *resulted in marked and appreciable yield decreases*. When 10 kg. copper per hectare were given the responses to nitrogen were better, and the growth inhibition only started when about 100 kg. nitrogen were given. When the field had received 200 kg. copper the response to nitrogen was much better and the yield doubled, the chance of growth inhibition in practice is much decreased. In Table 48 the nitrogen and copper relationship in grass is shown in a little more detail.

TABLE 48

The relationship between nitrogen and copper soil dressings on the copper content of Lolium perenne. From MULDER [199]

NITRO- GENOUS FERTILIZER,	COPPER CONTENT IN P.P.M. DRY MATTER				
		5 MG. CuSO ₄	20 MG. CuSO ₄	50 MG. CuSO ₄	200 MG. CuSO ₄
NH ₄ NO ₃ , G./POT	NO Cu SUPPLIED	PER POT	PER POT	PER POT	PER POT
0.5	8.8	9.0	6.6	7.1	13.1
1	6.8	7.2	7.3	8.7	9.3
2	6.0	8.0	7.3	6.8	7.8
4	4.2	5.2	6.0	7.1	11.5

The grass was grown in pots on a copper-deficient soil, and cut at the hay stage.

Increasing levels of nitrogenous fertilizers induce a decrease in copper concentration in the grass. The addition of copper to the soil, even in the presence of these fertilizers, increases the copper content of the vegetation. Many of the lighter western European soils show this type of response.

The fact that high levels of nitrogen can result in decreased copper levels in vegetation is of great practical significance in animal production. There is little reason to doubt that copper deficiency is increasing [208, 254] in areas such as northern Germany, and that stock are suffering accordingly. That low levels in copper, induced by nitrogenous fertilizers, have an immediate effect on the animals grazing on them is clearly shown in Fig. 74. The copper content of the blood serum of animals grazing the heavily fertilized field falls to very dangerously low levels [44], which is well below the safety level of 0.65 mg. copper/l. serum that is regarded as necessary in Holland.

Again, the loss in nutritional value of deficient vegetation, which was discussed in Chapter VI, must be borne in mind when stock are

discussed, as the proteins and vitamins of the pastures may become depleted as well. In maize heavy nitrogen fertilization results in an increased crop, but the nutritionally poor protein zein is also increased to a marked extent [268]. The influence of manganese upon the zein concentration in maize has already been mentioned (see page 71), and the fact that its application decreases the amount of this undesirable protein has been stressed.

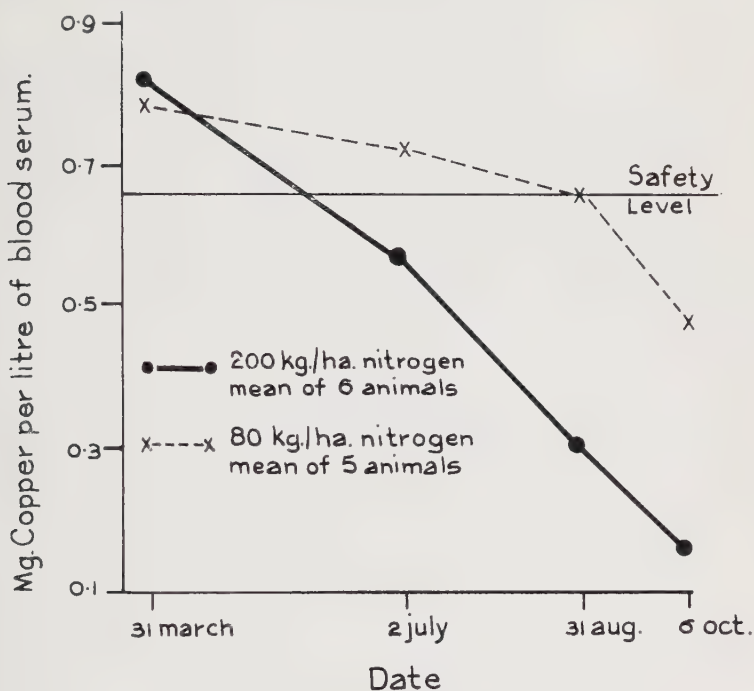


FIG. 74

AFTER BOSCH [44]

Relationship between the blood serum copper level of heifers and the nitrogen fertilization of pastures

One of these pastures had received 200 kg./ha. [178 lb./acre] of nitrogen annually for 16 years, the other, 80 kg./ha. [71 lb./acre] for the same time. In both pastures the serum copper of grazing heifers fell below the recognized safety level of 0.65 mg./litre by the end of the season, but on the heavily fertilized field it dropped to dangerously low levels.

Grazing these fertilized pastures caused the animals to become copper deficient.

Phosphatic fertilizers

In many parts of the world low levels of available phosphorus constitute the greatest limiting factor in agriculture, and phosphatic

fertilizers are essential for increasing the fertility of the soil and maintaining it. But just because this is so, there is a temptation to give overdoses of this fertilizer, especially in market gardening and other forms of very intense cultivation such as fruit or flax farming. As a result, certain conditions, such as phosphate-induced zinc deficiency, are very common in greenhouse crops like tomatoes. Again this kind of disturbed nutrition is more common on modern intensively cultivated farms than it is on carelessly run and rather neglected ones.

Bingham and his co-workers [39, 40], working on citrus, clearly demonstrated the influence of phosphatic fertilizers on the trace elements in citrus. Growing their plants in pots, they showed that the addition of phosphates decreased the absorption of boron, copper, and zinc, even though the addition decreased the soil pH. High levels of phosphate appear to promote manganese absorption, and do not alter iron uptake. An example of their results is shown in Table 10 (page 58). These workers examined nineteen soils and found that, regardless of source, excess phosphate resulted in acute copper deficiency, Table 49. On acid soils zinc and boron uptake was retarded

TABLE 49

The leaf copper content of sour-orange seedlings in relation to the nature of the soil and the rate of phosphate fertilization. From BINGHAM AND GARBER [39]

SOIL TYPE	pH	Ca(PO ₄) ₂ .H ₂ O, LB. P/ACRE	COPPER
			CONTENT OF LEAF, P.P.M.
Olivenhain loamy sand	4.65	100	5.5
	4.85	900	1.6
Escondido silt loam	6.10	100	5.3
	6.00	900	1.8
Moreno sandy loam	7.50	100	3.3
	6.81	900	1.1

Heavy dressings of phosphate depressed the leaf copper content on all three soils.

On soils with a low initial boron or zinc status, heavy phosphate dressings may induce deficiencies of these elements as well.

and that of manganese and molybdenum promoted. On alkaline soils excess phosphate decreased molybdenum uptake.

That excess phosphate induces a zinc deficiency is widely recognized [66, 173, 191], and a typical state of affairs is shown in Table 50, where the application of heavy doses of superphosphate result in a marked decrease in the zinc content of oats [245]. The data on flax is very similar [173].

Of particular importance to grazing animals is the fact that heavy

TABLE 50

The influence of superphosphate applications to the soil upon the zinc and phosphorus level in oats. From ROGERS AND WU [245]

QUANTITY OF SUPER- PHOSPHATE APPLIED IN, LB./ACRE	SOIL pH	YIELD PER POT, DRY MATTER, G.	ZINC CONTENT, P.P.M. DRY MATTER	PHOS- PHORUS CONTENT, % DRY MATTER
0	5.64	8.7	30.1	0.24
500	5.68	9.2	17.0	0.61
1,000	5.59	8.9	16.6	1.06
1,500	5.45	8.6	17.9	1.40

The superphosphate depresses the zinc content of oats, although it hardly influences the pH. At the same time the phosphorus content rises appreciably.

applications of phosphate increase the molybdenum content of the vegetation, sometimes as much as ten-fold [291]. As was mentioned, in areas like Britain a great deal of the copper deficiency in cattle is induced by high levels of molybdenum in the diet, and so the fact

TABLE 51

*The influence of phosphate application upon the copper, molybdenum and zinc content of tomato leaves, grown in pots of three different kinds of soils. From CANNELL *et al.* [64]*

PHOSPHATE ADDED PER POT, G.	SHOOT WEIGHT, G.	P.P.M. DRY MATTER IN LEAVES			Cu/Mo RATIO
		Zn	Cu	Mo	
OLIVENHAIN LOAMY SAND pH 4.6					
2	47	147	4.5	0.57	7.9
10	52	34	3.5	0.50	7.0
26	55	28	3.1	0.85	3.6
50	61	26	3.3	0.94	3.5
ENCONDIDO SILT LOAM pH 5.7					
2	64	30	12.3	0.98	12.5
10	77	21	8.9	1.68	5.3
26	83	21	4.3	1.94	2.2
50	88	19	4.8	2.00	2.4
MORENO SANDY LOAM pH 7.6					
2	27	30	8.0	3.08	2.6
10	37	21	9.0	2.57	3.5
26	43	18	7.1	2.02	3.5
50	54	15	7.5	1.67	4.5

This experiment clearly demonstrates that even within the range of phosphate applications which result in increased growth, the zinc and copper contents of the leaves are depressed. The molybdenum uptake is enhanced by phosphates on acid soils, but decreased on alkaline ones. The Cu/Mo ratio is seriously disturbed.

that excess phosphates can not only decrease the copper content of herbage but also increase the level of the molybdenum is of topical interest, as the fertilizing of pastures, including the application of phosphates, is becoming very widespread.

That these variations in mineral composition of the plant are not confined to one soil type is clearly shown in Table 51, where acid and alkaline soils were used to grow tomatoes. On the acid soils application of phosphates decreased the copper:molybdenum ratio very markedly [64].

Potassium fertilizers

The influence of high levels of potassium on mineral imbalance is seldom studied, yet in the U.S.A. 'alfalfa yellows' (boron deficiency) following potash applications is sufficiently well known for certain states to incorporate boron into their potash fertilizers. The 'yellows' usually respond to boron treatment. In soyabeans potash fertilizers often depress the growth and the yield, but, in practice, if 2 lb boron are mixed with 2 million lb of soil there is sufficient boron present to enable soyabeans to grow at all levels of potassium that are likely to be supplied.

Fig. 23 (page 59) graphically depicts this potash-induced boron deficiency in soyabeans. Woodruff *et al.* [333] showed that potassium

TABLE 52

The influence of various levels of potassium in the soil, with and without added boron, on the composition of soya-bean leaves. From WOODRUFF et al. [333]

SOIL:		COMPOSITION OF LEAVES, DRY BASIS							
SATURATION WITH POTASSIUM, %	WITHOUT BORON				WITH BORON ADDED TO SOIL				
	B, P. P. M.	K, %	Ca, %	Mg, %	B, P. P. M.	K, %	Ca, %	Mg, %	
0	11	0.4	1.4	1.5	75	0.4	1.6	1.4	
0.5	42	0.5	1.2	1.1	78	0.3	1.7	1.2	
1.0	24	0.8	1.1	0.7	47	1.2	1.4	0.9	
2.0	34	1.0	0.8	0.6	54	1.5	1.4	0.8	
4.0	11	2.1	0.7	0.4	50	1.6	1.1	0.7	
8.0	6	2.1	0.6	0.3	49	2.7	1.3	0.7	
12.0	6	2.2	0.6	0.4	39	2.6	1.3	0.8	
16.0	12	2.2	0.4	0.4	43	3.0	1.1	0.6	
20.0	Trace	3.1	0.3	0.4	—	3.0	1.0	0.8	

The potassium antagonizes the boron, and increasing the potassium level of the soil decreases the boron content of the leaves. In the absence of adequate supplies of boron in the soil, increasing potassium levels result in marked depression of calcium and magnesium absorption.

depressed the level of boron in the leaves when boron was not supplied to the pots, but in the presence of adequate supplies it did not influence the leaf content of boron in soyabeans. The fact that the boron also influenced both the calcium and the magnesium content of the leaves is clearly shown in Table 52. In its absence increasing concentrations of potassium result in decreasing levels of both calcium and magnesium, while in the presence of boron this was far less marked.

Some other problems

It is thus quite clear that over-application of the main fertilizers can and does result in serious imbalances of mineral nutrients in agriculture. The cases that have been cited make it quite clear that one can have too much of a good thing. And at our present levels of agriculture we must bear in mind not only the need for one or other essential element, which we are not so likely to forget, but the importance of *balanced* nutrition. Otherwise in our efforts to solve one problem we may quite unwittingly and unnecessarily develop another. This is very clearly shown by the events that took place in Holland after the Second World War.

The war destroyed the Dutch economy and as soon as it was over every effort was made to build up the agricultural industry for which Holland had been justifiably famous. So very many farmers started to give very heavy applications of nitrogenous, phosphoric, and potash fertilizers to their soils in the belief that this would rapidly build up the fertility of the fields again. Authorities such as Grashuis [117] tried to warn against this procedure, as it would upset the nutritional equilibria of the soil and vegetation. But their worst fears were realized when the milk production, reproduction, and health of the farm animals was seriously disturbed. On small, heavily stocked farms, where much manure was available, the level of potassium and of phosphorus in the soil reached such heights that many farmers were advised to sell their manure until the nutritional balance of the soils had been re-established. In the 450 farms investigated in detail 89½ per cent had levels of phosphorus that were too high, and only 9·8 per cent were normal. 67·8 per cent had too much potassium, and 30·1 per cent were normal. Yet 85·5 per cent of the grasslands were deficient in copper, and 79·5 per cent had cobalt levels of less than 0·30 mg. Co/kg. soil. These conditions resulted in marked copper, manganese, and cobalt deficiency in grazing animals, all of which had to be treated. There was no doubt at all about the serious consequences of the disequilibria brought about by unbalanced fertilization.

The conditions prevailing in Holland may not be too common, but even in an industry like the poultry industry, where more animals

are now produced under very artificial conditions than ever before, attention must be paid to mineral balance. High levels of phosphorus and of calcium in the diet result in a marked increase in the manganese requirements of the birds, and unless this is supplied, deficiency sets in [62].

The antagonisms that exist between nutrients must be accepted and their implications appreciated. A fine example of the way in which an antagonism between a trace element and one of the major element fertilizers can be successfully exploited is described in the work of Staker [283]. Peat soils which were unproductive due to toxic levels of zinc were economically 'cured' by liming and brought under cultivation. Some details are given in Table 53. Other toxicities can be treated in a similar way, and use can be made of this property of antagonism in both animal and plant production.

TABLE 53

Liming as a cure for zinc toxic soils, using spinach as the test organism.

After STAKER [283]

SOIL TREATMENT	AVERAGE FRESH WEIGHT PER POT, G.	AVERAGE DRY WEIGHT PER POT, G.	ZINC CONTENT, P.P.M. DRY MATTER
<i>Control: normal soil + fertilizer</i>	64.47	4.964	49
<i>Zinc-toxic soil</i>			
Fertilizer only	3.31	0.392	783
Ca(OH) ₂ to pH 7	53.41	4.448	721
Ca(OH) ₂ 4 tons/acre	55.65	4.898	577

The fertilizer treatment which all pots received was equivalent to 1,000 lb/acre 4 : 16 : 20 NPK.

The liming antagonized the zinc so successfully that normal yields could be obtained, although the zinc content of the spinach still remained very high.

There is a possibility that the contents of this chapter may be regarded as an attack on the fertilizer industry, but nothing could be farther from the truth. Modern intensive agriculture is completely dependent on the artificial fertilizers that are present in so many forms on the markets of the world. If production is to go on increasing as it is doing these fertilizers will be one of the main factors involved. But it would be shortsighted to ignore the fact that they can be abused, and that ignorant use of them can result in serious damage.

If production and quality is to be improved *balanced nutrition must be maintained*. All essential elements must be present and available in adequate amounts. If a copper shortage exists, increased nitrogen fertilization is not only useless—it is uneconomic and senseless. But once the deficiency is made good, then increasing the nitro-

gen level will probably result in bigger and better crops. Wrongful fertilization, which results in copper-deficient animals, is as little in the interest of the nitrogen or phosphate industry as it is in the farmer's. It is up to the fertilizer industry to warn their customers against the abuse of their products and to see that they receive good and competent advice on imbalance problems when they are suspected. The fact that *total balanced nutrition* is stressed does not constitute either an attack on the artificial fertilizer industry or a threat to it. It is in their own interest to appreciate this, and to recognize that to pursue a policy of selling the maximum of fertilizer irrespective of its effects is both anti-social and ultimately very bad business.

Conclusion

Essential nutrients are absolutely necessary for normal development and existence. This is clearly implied in the term 'essential'. But it is not always appreciated that far more than mere presence or absence of these nutrients is involved. The essential trace elements are a very fine example of the role such essential nutrients can play in living matter. Nutrition is involved in virtually all living processes to some extent, and the contention that we are what we eat is not just a smart cliché, but a very profound concept. A study of the biology of trace elements enables a coherent, but certainly not a comprehensive, picture to be built up, depicting the multitudinous ramifications of nutrition. Such a picture is not nearly so readily constructed in the realm of macro elements or of organic nutrients, which are vaster and more complex. But the criteria of *essentiality* stressed here also apply in those fields.

Balanced nutrition

It cannot be over-emphasized that for proper development *balanced nutrition* is of the utmost importance. In living matter it is impossible to obtain *absolute deficiencies* of any essential constituent, and *relative imbalances* are found. There is no balance of nutrients that is optimal for all species, and in fact there is none that is optimal for any given species. The environmental factors, such as climate and the nature of the soil, can and do alter the minerals that a plant and animal needs. The ratio of mineral nutrients required on a sandy tropical soil for maize is quite different from that on a heavy soil in central Asia. There are no *absolute* limits in nutrition, be they for deficiency, excess, or optimal growth. The environment must be considered when the optimal balanced nutrition of an organism is being assessed.

Trace-element imbalances

From a practical point of view, it is essential to grasp the fact that trace-element imbalances do exist on a very extensive scale, and that there are very real trace-element problems. It is only when this has been properly appreciated that really effective measures can be taken to remedy the 'problems', which are so widespread as to be a very normal part of our environment. But this will only be the case when the significance of *latent* deficiencies and excesses is grasped.

Practical agricultural considerations

There is no doubt that the rapidly increasing cost of agricultural labour and equipment will result in an effort to make the land more productive. Outmoded agricultural techniques will be replaced because they are uneconomical. Stress will be laid upon increasing productivity, but unless attention is paid to the *quality* of produce, this can result in very harmful consequences, such as copper-deficient pastures, for instance, as a result of *unbalanced* nitrogen fertilization. Certainly, agricultural production must continue to rise with new strains and varieties produced by breeders, new agricultural techniques, and new fertilizer practices, which will pay attention to supplying *all the essential mineral* elements required.

Future in agriculture

There is every reason to believe that great changes will take place in agricultural practice in the not so distant future. One of the most interesting will almost certainly be that plants will be fertilized in such a way as to be adapted to their environment. As was shown, the trace elements play an important part in governing the water relations of plants, as well as their resistance to damage by frost, heat, or wind. Nutrients can hasten or prolong the time required for fruiting, and weather is not the sole determining factor involved. The use of copper fertilizers to evade the dangers of drought may become standard practice in arid regions. While the principles required for this type of constructive fertilizer practice have been established, it is not yet possible to conjecture on how they will be applied, or the difficulties that may be encountered.

It is very probable that increasing interest will be taken in the fact that proper nutrition can be used to combat fungal infections in plants and worm parasites in animals. Treatment to increase resistance to infection will undoubtedly be economic, as losses due to these causes are very high indeed. That does not imply that fungicidal sprays, etc., will be unnecessary, but only that the need for them would be restricted.

Need for research

It is quite clear that there is a great need for more research work to be undertaken, if the information that we possess is to be adequately exploited. Nor is it only a need for agricultural research, where the behaviour of plants and animals under farming conditions is the main concern. There is also a need for 'pure' research, upon which agriculture depends to such a great extent.

An improved knowledge of plant physiology is urgently required. Work like Rademacher's [229], which shows that adaptation to copper

deficiency consisted in an ability to absorb this element rapidly, is of the utmost value to geneticists who wish to breed such desirable characteristics into plants. These breeders must have this type of information if they are to produce new strains rapidly. Or again, it is clear that investigations into the influence of trace elements upon alkaloid production, cf. Fig. 30 (page 78), could be of ultimate commercial interest. This type of study on essential oils or other valuable products could be very rewarding.

Another branch of pure research that is of great potential importance is soil microbiology. Is it possible to influence soil breakdown and rock weathering appreciably, and so influence the nutritional status of the soil? If it is, there is no doubt that trace elements will play an important part.

While new chemical and physical techniques have been responsible for the very rapid advances in biology that have taken place, it is also clear that other methods are urgently needed. One of the greatest practical difficulties encountered today is for simple, accurate, and reproducible techniques for determining *biological requirements*. The present methods seldom do this.

It is likely that in the field of cytology studies will result in bridging the gap between the purely biochemical studies of the enzymologist and physiological work. This would lead to a very important advance in our knowledge and understanding of living systems.

Health

Nutrition is of the utmost importance in maintaining health in plants, animals, and man. In the case of animals it is unfortunate that many farmers still consider unthrifty animals as almost a normal state of affairs, and robust health as something that is very desirable, but, alas, not too common. While there are many causes of unthriftiness, thousands of animals are below par because their mineral requirements have not been met. As these conditions are generally not acute, little is done to remedy them. Yet lack of well-being due to inadequate supplies of trace elements is readily and cheaply curable in most cases. There is little doubt that in a large number of areas, even the introduction of suitable grass mixtures* would result in pastures that supplied the animals' needs. As is well known, different pasture grasses contain appreciably different levels of trace elements. Some examples are shown in Table 54. And the widespread iodine, copper, and manganese deficiencies can be readily combated. It must be appreciated that sub-optimal health, and the resultant decrease in fertility, etc., are luxuries that are sub-economic in modern farming.

* Grass mixtures must be understood to include legumes and other herbs.

In human health iodine and iron pose the main problems, and their deficiencies do, in fact, take a great toll of health and happiness. Again, unhealthy people have a much reduced working capacity, and so this is important from an economic point of view as well. Human health problems are not so readily solved, and the existence of goitres and anaemias not due to simple deficiencies pose some very real practical problems in their treatment. Nevertheless, the diets are very important, and the mineral composition of foods must be considered seriously if normal health is to be promoted.

TABLE 54

Trace-element content of some pasture grasses and herbs grown in M'Vuazi, Congo Republic. Quantities are expressed in mg./kg. dry matter

SPECIES	Fe	Cu	Co	Mn
<i>Hyaparrhenia diplandra</i> (Hack) Stapf.	940†	6.5	0.141	79
<i>Melinis minutiflora</i> P. Beauv.	648*	7.4	0.188	127
<i>Schizachyrium platyphyllum</i> Stapf.	382	29.1†	0.149	481†
<i>Brachiaria ruziziensis</i> Germain et Evrard.	257	4.7	0.112	36
<i>Chloris gayana</i> Kunth.	402	3.8	0.117	60
<i>Digataria Umfolozi</i>	579	19.2†	0.244	355†
<i>Setaria splendida</i> Stapf.	168	4.25	0.680†	128
<i>Stylosanthes gracilis</i> H.B.K. (legume)	527	11.7	0.227	218

* Appreciably higher than average.

† More than 30 per cent higher than average.

The fact that trace elements play a part in such conditions as cancer, atherosclerosis, and hypertensive states makes them particularly interesting. However, their exact importance in these conditions must be established by further research. It is only clear that they are associated with an alteration in the balance of trace elements, and that as these play such an important part in the enzymatic activity of the cell, they might be expected to be seriously involved in such conditions.

In conclusion, it must again be stressed that trace-element nutrition is only part of the general problem of nutrition. The other essential nutrients were not discussed, because they fall outside the scope of this book. The material of this volume must be seen in a context of the other essential nutrients, which are absolutely necessary for normal development. Only then is it possible to get a glimpse of the importance of nutrition and its many fascinating implications — a glimpse that shows to what a large extent health of mind and body is dependent upon the food we eat and upon the trace elements which they contain.

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Further reading

A brief list of books, readily available, for further reading, and for obtaining more detailed references than are contained in this book.

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Discusses methods for the determination of the nutritional requirements of plants. Contains extensive tables of the element contents of plants as obtained from the literature. Extensive bibliography.

MONIER-WILLIAMS, G. W., *Trace Elements in Food* (John Wiley, London and New York, 1950), 511 pages.

Well-written and interesting book discussing the trace-element content of foods. Full of unusual information not readily available elsewhere. Thirty elements are discussed.

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Deficiencies and excesses of minor and major elements are discussed. These conditions in all parts of the world, their similarities and dissimilarities are described, and a brief general discussion is included.

SCHARRER, K., *Biochemie der Spurenelemente* (Paul Parey, Berlin and Hamburg, 1955), 404 pages.

This book incorporates references to much scientific work which does not normally appear in English. While very comprehensive in its scope, it lacks an index, and this reduces its value as a reference book. It does not deal with complex biochemistry to any appreciable extent, and can be read by non-chemists.

To date, no English translation of this book has been published.

STILES, W., *Trace Elements in Plants* (Cambridge University Press, 1961), 249 pages.

Starting with an historical introduction, and the techniques involved in trace-element investigations, this book describes the plant diseases due to the deficiency and excess of these elements. Then some of the physiological problems associated with them are discussed. A brief chapter on their role in animals is also included. Good bibliography.

SWAINE, D. J., *The Trace Element Content of Soils* (Commonwealth Bureau of Soil Science, Technical Communication No. 48, Commonwealth Agricultural Bureau, 1955), 157 pages.

Information gleaned from the literature of the world is tabulated to give the trace-element contents of the various soils which have been analysed. Extensive bibliography.

UNDERWOOD, E. J., *Trace Elements in Human and Animal Nutrition* (Academic Press, New York, 1956), 430 pages.

The only book of its kind at this level. Well written and informative.

it covers an extensive range of phenomena and is well indexed and contains a large number of references. It is the ideal book to introduce the reader to trace-element nutrition in animals. Contains relatively little about human nutrition, as very little was known at the time of publication.

VOISIN, A., *Soil, Grass and Cancer* (Crosby Lockwood and Son Ltd., London, 1959), 302 pages.

A most stimulating book, showing the implications of mineral deficiencies upon the health and well-being of animals and man. A very well-presented argument showing how all living matter is dependent on the soil, and showing how a disease can be linked to soil or water deficiencies even in modern times. Good bibliography.

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This book includes a large number of colour plates showing the visual symptoms of deficiencies and excesses that occur in normal temperate crop plants. It discusses the value of these symptoms.

WILLIAMS, R. DORRINGTON, *Minor Elements and Their Effects on the Growth and Chemical Composition of Herbage Plants* (Mimeographed publication No. 1/1959, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire), 68 pages.

A short but informative and clearly written booklet, referring not only to the role of the minor elements in plant nutrition but discussing factors which affect their content in herbage, such as climate and botanical composition of the vegetation, etc. Practical applications of the trace elements are well discussed. Extensive bibliography.

More specialized reading

Bibliography of the Literature on the Minor Elements and their Relation to Plant and Animal Nutrition, Volumes 1-4 (Compiled and published by the Chilean Nitrate Educational Bureau Inc., New York, 1948 onwards).

Comprehensive abstracts of the world literature. Invaluable reference work.

Encyclopaedia of Plant Physiology, Volume IV, Ed. RUHLAND, W. (Springer Verlag, 1958).

Comprehensive articles by a number of authorities dealing with various aspects of mineral nutrition are contained in this volume. Many of the chapters are written in German and are not translated into English, but a bilingual subject index is of great value.

SCHWEIGART, H. A., *Vitalstoff-Lehre, Vitalstoff-Tabellarium* (Hans Zauner Verlag, München, 1962) 230 pages.

This book, written in German, has many valuable tables giving information about trace elements in organisms, foods and enzyme systems. It also includes similar tables on vitamins, hormones, fats, proteins and carbohydrates.

The following scientific periodicals and reviews frequently contain up-to-date articles concerning trace elements, or abstracts of them:

- (1) *Biological Abstracts*.
- (2) *Chemical Abstracts*.
- (3) *Fertiliser Abstracts*.
- (4) *Annual Review of Plant Physiology*.
- (5) *Plant and Soil*.
- (6) *Soil Science*.

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